DIGESTION AND UTILIZATION OF RAPESEED MEAL BY THE GROWING PIG

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by

Rodrigue J. Gregoire

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ABSTRACT

Six-week old castrated male pigs were used in three experiments designed to investigate the factors responsible for the low apparent digestibility of rapeseed meal (RSM). Results of Experiment I indicated that method of processing (solvent, prepress-solvent, or expeller), variety of rape (B. napus, B. campestris, and B. napus cv. Bronowski) and year of harvest (1967 and 1968) had no significant effect on protein and amino acid digestibilities and efficiency of nitrogen retention of RSM. Apparent protein digestibilities for 12 samples of RSM from 5 different processors ranged from 71.0 to 77.9%. Apparent digestibilities of the individual amino acids varied from 69.0 to 84.6%. In general, digestibilities were highest for glutamic acid, histidine and arginine. In Experiment II digestibility of RSM was compared with that of other protein sources. Coefficients of apparent protein digestibility for three samples of RSM averaged 75.8 compared to 91.8 for casein (Cas), 79.3 for autoclaved casein (Aut.Cas.), 84.2 for soybean isolate (Soy) and 80.8 for zein (Ze). Protein digestibilities based on digesta from the ileum of pigs killed approximately 4 hours after a meal of the test diets bore little relationship to digestibilities based on feces collected from these pigs, particularly in the case of Soy and Ze. Apparent digestibility coefficients based on ileal digesta were 73.2, 86.3, 78.2, 70.3, and 60.8 for RSM, Cas, Aut.Cas., Soy, and Ze, respectively. In general, digestibilities of the individual amino acids followed those of the protein sources although there was considerable variation in digestibility

coefficients among amino acids. Digestibility coefficients tended to be highest for glutamic acid, histidine, and arginine, and lowest for methionine and alanine. Digestibility coefficients based on digesta from the ileum agreed fairly closely with those based on feces except for proline, glycine, glutamic acid, aspartic acid, threonine, and serine where digestibility improved markedly between the ileum and feces, particularly with Soy and Ze which suggests that caution must be exercised in the interpretation of amino acid digestibility data based on fecal analyses. Partial removal of the hull fraction from RSM by air classification (Exp.III) resulted in a significant ($P<0.05$) improvement in protein digestibility; coefficients of apparent digestibility averaged 75.7 for the three regular RSMs and 79.8 for low-fiber RSM prepared from these meals. Fiber did not affect the digestibility of soybean protein; coefficients of protein digestibility were 83.4 for soybean meal (44% protein) and 83.5 for soybean isolate. No significant differences were found among the RSM and soybean sources for protein digestibility coefficients in the mid jejunum and ileum. However, a significant ($P<0.05$) improvement in digestibility was found between the ileum and feces for pigs fed soybean protein, either as meal or isolate, and two of the low-fiber RSMs. These results suggest that the hull fraction in RSM interfered with fermentation of the nitrogenous components in the large intestine or increased the rate of transit through this site. Besides, efficiency of nitrogen retention with RSM expressed as % N intake or percent N absorbed was significantly ($P<0.05$) higher than that of soybean protein. In vitro pepsin, pancreatin, and pepsin-pancreatin digestion of RSM (cv. Oro), Cas., Aut.Cas., Soy, Ze, and soybean meal bore little

relationship to the *in vivo* digestibilities of these proteins. The results of these experiments suggest that the lower apparent protein digestibility of RSM than of soybean meal was the result of the greater fermentation of the latter in the large intestine.

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Chapter I

GENERAL INTRODUCTION

The 1971-72 Canadian production of rapeseed was 98.5 million bushels compared to only 10 million bushels a decade earlier. With the growing popularity of rape as a crop in Canada, rapeseed meal has become a very economical source of protein. The use of rapeseed meal as a protein source in animal diets, however, is complicated by the presence of potential goitrogenic factors (glucosinolates). In addition, recent experiments with rats and pigs have indicated a lower digestibility of rapeseed meal protein compared with casein and soybean protein. The reason for the lower digestibility of this protein has not been systematically investigated. Thus the principal objective of the present study was to resolve some of the factors responsible for the relatively poor digestibility of rapeseed protein as compared to other protein sources. The first experiment studied the influence of method of processing and variety of rape on the digestibility and utilization of rapeseed protein. The second experiment compared the progress of *in vivo* digestion of protein from rapeseed meal with that of casein, autoclaved casein, isolated soybean protein and zein in growing pigs. Amino acid patterns in digesta at different levels of the intestinal tract were compared for pigs fed the various protein sources in an attempt to estimate the extent of protein digestion by the time digesta reaches the ileum and to establish the influence of microflora on the apparent availability of the individual amino acids. The possibility exists that the lower digestibility of rapeseed meal is due to less fermentation of this

protein than other types of proteins in the large intestine. A third study dealt with the effect of removal of the hull fraction in rapeseed meal on the digestibility of this protein. In addition, in vitro pepsin and pancreatin digestions were carried out for the various protein sources to determine if the lower digestibility of rapeseed meal was related to rate of hydrolysis by proteolytic enzymes.

Chapter II

REVIEW OF LITERATURE

2.1 Studies on the digestibility and utilization of rapeseed meal

2.1.1 Introduction

The nutritional status of rapeseed meal (RSM) was reviewed by Bell (1955) and he concluded that rapeseed meal was a poor protein source because of the presence of goitrogenic factors in this meal. However, little information was presented on the digestion and metabolic utilization of rapeseed meal by domestic animals. Since that time considerable information has been accumulated on various aspects of the nutritive value of rapeseed meal. The present chapter will therefore deal with some of the factors, i.e. feed intake, digestibility, goitrogenic substances and processing, which affect the utilization of rapeseed meal in different species.

2.1.2 Effect of feed intake on apparent utilization of rapeseed meal

Bell (1955) indicated that appetite depression, poor weight gain and reduction in feed efficiency generally accompanied the feeding of RSM to different species. In subsequent trials with mice, Bell (1957) and Belzile *et al.* (1963) reported highly significant correlations ($r = 0.71$ and 0.67, respectively) between the poor weight gain and low intake of diets containing RSM. In rats, lower feed efficiency with RSM was paralleled by a two-fold decrease in feed intake (Goering *et al.*, 1960). Drouliscos and Bowland (1969) also reported that the lower protein efficiency ratio (PER) with RSM was related to some extent to

the lower feed intake of rats fed RSM. Protein efficiency ratios averaged 2.0, 1.9, 2.4 and 2.3 for solvent extracted RSM, prepress-solvent RSM, soybean meal, and casein whereas the corresponding nitrogen intake was 103, 119, 140 and 130 gm. Feed intake also tended to be depressed in pigs with inclusion of RSM in a basal diet. Schuld and Bowland (1967) found that feed intake, rate of gain and feed conversion of pigs up to 110 pounds in weight were poorer when 50 to 100% of the soybean meal in the diet was replaced by rapeseed meal. Data from Bowland (1971) indicate that a depression of approximately 2% in feed intake can be expected for each 1% of dietary rapeseed meal added even when adjustment was made for energy (DE) and protein level.

There is evidence that the glucosinolate content is partly responsible for the lower feed intake and weight gain of animals fed rapeseed meal. Clandinin et al. (1959) reported that high glucosinolate rapeseed meal (Hi RSM) was more detrimental to feed intake and growth of chickens than low glucosinolate RSM (Lo RSM). Ten percent of Argentine RSM (Brassica napus), a high glucosinolate variety, depressed feed intake and growth of chicks while as much as 15% Polish RSM (Brassica campestris) had little effect. Similarly Oliver et al. (1970) reported that a high thioglucoside RSM (Hi RSM) significantly reduced feed intake and weight gain of rats as compared to diets containing either Bronowski rapeseed meal (a low thioglucoside RSM) or casein. The feed intake of mice fed Bronowski rapeseed meal was equivalent to that of mice fed soybean meal (SM) even when Bronowski constituted up to 20% of the total diet (Bell et al., 1971). By contrast two sources of Hi RSM, Target and Yellow Sarson, depressed feed intake and weight gain even

when adjustment was made for digestible energy (DE) intake. However, the relationship between total glucosinolate ingestion and observed or DE adjusted weight gain was rather poor ($r = -0.57$ and -0.21, respectively). This poor relationship between DE adjusted weight gain and glucosinolate content of RSM suggests that a considerable portion of reduced weight gain is unexplained. Other factors such as type and level of glucosinolate in the diet or degree of glucosinolate cleavage in the gastrointestinal tract might explain the lower weight gain with RSM.

The lower weight gain with RSM, compared to other protein sources, is usually associated with a depressed feed intake. The thioglucoside content of most varieties of RSM appears to be responsible for the reduction in feed intake and in turn depressed weight gain and poor efficiency of feed utilization by animals fed RSM.

2.1.3 Digestibility of rapeseed meal

Numerous experiments have been carried out during the past decade on the digestibility of RSM in rats and pigs. Most of the reports indicated consistently lower digestibility coefficients for protein, dry matter, fiber and energy of RSM. In rats (Drouliscos and Bowland, 1969) true nitrogen digestibility of solvent RSM or prepress-solvent RSM was found to be 78.7±1.2 and 77.9±2.7 respectively, compared to values of 90.4±0.9 for soybean meal and 96.0±0.6 for casein. Similarly Oliver *et al.* (1970) reported that the apparent digestibility of low and high thioglucoside RSM by the rat was similar (80 and 82% respectively)

but considerably lower than that of casein (94%). The true digestibility coefficient of RSM for colostomized chickens (Tao et al., 1971) was lower (78) than those reported from the same laboratory (Flipot et al., 1971) for casein (96), fishmeal (95), soya protein (93) and zein (85). Cho and Bayley (1970) also found the digestibility coefficients for protein, dry matter, gross energy and crude fiber to be 8-12% lower for RSM than for soybean meal when 60 kg. pigs were fed semi-purified diets containing either rapeseed meal or soybean meal as the sole source of protein. Energy digestibility and true protein digestibility of RSM (72 and 84%, respectively) were similar to the values found for oats (69 and 82%), but lower than those for soybean meal (82 and 88%), barley (84 and 92%), fishmeal (89 and 95%), wheat (90 and 96%) (May and Bell, 1971). These values agree with the observations of several groups (Saben et al., 1971a, 1971b; Bayley et al., 1969; Manns et al., 1963b; Hussar and Bowland, 1959b) who have found that the addition of 10 to 25% of RSM to a basal diet decreased the overall digestibility and the metabolizable energy yield of the diet.

Method of processing used in oil extraction of rape apparently has little effect on the digestibility of nitrogen, energy and dry matter by rats and pigs. Protein digestibility of expeller RSM (Hussar and Bowland, 1959b) was approximately the same as that of solvent RSM (Manns et al., 1963b). Drouliscos and Bowland (1969) reported similar protein digestibilities for prepress-solvent and solvent extracted RSM and Saben et al. (1971a) found no differences in protein digestibility among 12 samples of RSM processed by expeller, prepress-solvent or solvent processes. Mean apparent coefficients of protein digestibility

for expeller, prepress-solvent and solvent RSM were 75.0, 74.6 and 76.3, respectively. The highest and the lowest digestibility values were observed for samples of solvent extracted RSM. These observations by Saben et al. (1971a) also suggest that thioglucoside level has little effect on digestibility of nitrogen and energy in RSM since the values for Bronowski RSM were similar to those for the other meal. Similar findings were reported by Oliver et al. (1971).

Little information is presently available on the influence of fiber content in RSM on the digestibility of this meal. Saben et al. (1971a) reported no difference in digestibility of nitrogen and energy among 12 samples of RSM containing from 13.7 to 16.0% of fiber. However, they suggested that the rather high crude fiber content of RSM and the nature of this fiber may have decreased the digestibility of the protein. It is possible that in RSM lignin is arranged and distributed in such a way that protein is trapped by the fibrous material and therefore prevents its rapid digestion by proteolytic enzymes. This is demonstrated by the fact that protein digestibility generally follows that of the dry matter and energy (Hussar and Bowland, 1959b; Manns et al ., 1963b; Bowland and Schuld, 1968; Bayley et al., 1969; Cho and Bayley, 1970; Saben et al., 1971a; Bowland, 1971). Digestibility of crude fiber in RSM is also considerably lower than that of soybean meal or a corn-soybean meal diet (Bayley et al., 1969; Cho and Bayley, 1970). Bayley et al. (1969) reported that steam-pelleting and regrinding RSM increased crude fiber digestibility from 37.8 to 56.9% as well as improving dry matter, nitrogen and energy digestibility by 2.5, 2.5 and 10% respectively. These observations support the suggestion that poor

digestibility of crude fibre is probably one of the factors responsible for the lower digestibility of dry matter, protein and energy observed with RSM.

2.1.4 Metabolic utilization of rapeseed meal

The fact that inclusion of RSM in the diet depresses feed intake and coefficients of digestibility for protein and energy raises the question of whether those factors alone account for the poor weight gain and nitrogen retention observed with animals fed diets containing RSM or whether the metabolic utilization of rapeseed meal is inferior to that of other protein sources. The possibility also exists that thyroid impairment and protein quality affect the metabolic utilization of the absorbed amino acids.

A. Nitrogen retention with RSM and other protein sources

Several researchers (Hussar and Bowland, 1959; Drouliscos and Bowland, 1968; Bowland and Schuld, 1968; Cho and Bayley, 1970; Wood and Stone, 1971) have suggested that the impaired nitrogen retention obtained with RSM is probably the result of the lower digestibility of protein, dry matter and energy. Hussar and Bowland(1959b) reported that the inclusion of 2 and 10% of RSM in a basal diet containing soybean meal, wheat and fish meal did not significantly alter the retention of absorbed nitrogen by pigs and rats although there was a reduction in the digestibility of dry matter, energy and nitrogen, especially with the 10% level of RSM. Bowland and Schuld (1968) also found that nitrogen retention, as percent of the N digested, was similar for pigs fed rapeseed meal to that of pigs fed soybean meal during the starting (52.2 and 55.5, respectively) and growing (42.5 and 40.8, respectively) periods. The authors concluded

that the slightly higher nitrogen retention with the soybean ration during the starting period was associated with the higher digestibility of this diet. This conclusion is supported by the observations of Drouliscos and Bowland (1968) and Cho and Bayley (1970). Drouliscos and Bowland (1968) found that the biological value (BV) of solvent and prepress-solvent rapeseed meals for the rat was similar to that of casein and soybean meal although net protein utilization (NPU) values were 5-14% lower for the two rapeseed meals than for soybean meal and casein during the growth period. The authors concluded that the lower nitrogen balance obtained with rats fed RSM was related to the lower feed intake and lower digestibility of these meals and not to impaired metabolic utilization of the protein. Cho and Bayley (1970) also found that percentage of absorbed nitrogen by pigs fed soybean meal (84.0±2.2) was not statistically different from that of pigs fed RSM (78.9±3.4) although less nitrogen was retained with RSM (61.9±3.3) than for the SM diet (73.9±2.9) when expressed as a percent of the total nitrogen ingested.

Thus the lower nitrogen retention observed with RSM as compared to other protein sources appears to be related mainly to lower feed intake and lower digestibility of dry matter, nitrogen and energy rather than impaired utilization of absorbed N. This conclusion is supported by the findings that potential goitrogenic factors have little effect on metabolic utilization of absorbed N.

B. Nitrogen retention and thyroid impairment

Thyroid impairment and hypertrophy has been observed in pigs (Nordfedt et al., 1954; Hussar and Bowland, 1959a), rats and mice (Kennedy and Purves, 1941; Hussar and Bowland, 1959a; Belzile et al.,

1963; Drouliscos and Bowland, 1969; Oliver et al., 1970) and chicks (Clandinin et al., 1959; Woodly et al., 1972) fed RSM. It is also well established (Bell, 1957; Manns et al., 1963a) that feeding RSM results in lower standard metabolic rates in rats and mice as well as a decrease in plasma bound iodine (Manns et al., 1963a). It has been postulated (Konigsberg, 1958; Manns et al., 1963a) that thyroid gland malfunction may give rise to an overall disturbance in metabolism and that inadequate thyroxine supply probably interferes with the normal protein synthesizing mechanism through a disruption of normal energy pathways.

Although the important role of thyroxine hormone in protein synthesis is well established (West et al., 1967) there is no evidence that goitrogenic substances inducing thyroid hypertrophy in animals result in less efficient nitrogen utilization. According to Bell (1957) mice fed diets (equal parts of soybean meal and linseed meal) containing 0.15% oxazolidinethione gained more slowly than mice fed RSM diets. Adjustment of gains for feed intake showed that the effect of the goitrogenic factor was related to appetite or palatability rather than to feed utilization. Bowland and Standish (1966) also reported that the addition of 0.05% thiouracil to a basal diet containing 13% of SM produced a similar thyroid hypertrophy in rats to that obtained with RSM although the presence of thiouracil did not appear to reduce nitrogen retention (as % of the protein intake). On the other hand Oliver et al. (1970) reported that N retention was lower with high thioglucoside RSM than with low thioglucoside RSM (Bronowski) even when differences in food intake were taken into account by covariance analysis. Similarly Bell et al. (1971) reported that growth was poorer for mice fed diets containing 20%

RSM from high thioglucoside varieties such as Target, Yellow Sarson, Nugget and Echo than for mice fed a low thioglucoside variety (Bronowski) RSM, even when gains were adjusted for digestible energy (DE) intake.

Thus, although a few reports (Oliver et al., 1970; Bell et al., 1971) suggest that the thioglucoside content affects the metabolic utilization of rapeseed protein, most observations indicate that the impaired nitrogen retention of animals fed RSM is associated with reduction in feed intake and nitrogen digestion rather than depressed utilization of digested nitrogen.

C. Effect of processing methods and variety on protein quality of RSM

The metabolic utilization of a protein is related to its amino acid composition and the biological availability of each amino acid to the animal. Thus the possibility exists that method of processing and variety of RSM affects the amino acid composition and availability and, in turn, nitrogen utilization.

Little information is available on the effect of methods of processing and variety of rape on utilization of RSM. Observations by Goering et al. (1960) suggested that solvent extracted RSM was superior to expeller meal as evidenced by the fact that gain on the solvent meal was almost double that on expeller meal. Data reported by Clandinin (1967) tended to substantiate this suggestion. The levels of several amino acids were lower in expeller RSM produced during 1956-1961 than in prepress-solvent RSM produced during the same years. The main differences were lysine (4.39 vs 5.31%), proline (5.71 vs 6.13%), arginine (5.09 vs 5.48%) and tryptophan (0.94 vs 1.21%). On the other hand, total lysine and "available" lysine were considerably higher in prepress-solvent RSM

produced during the years 1965-1967 than in meal produced during the years 1956-1961 (5.94 vs 5.31% and 5.68 vs 5.49%, respectively). Clandinin (1967) concluded that the improvement in the lysine content of RSM was probably attributable to the fact that more of the Polish type RSM (Brassica campestris) than Argentine RSM (Brassica napus) was grown in Canada during the years 1965-67 than the years 1956-61 and to reduced heat damage by prepress solvent processing during the years 1965-67. Thus it appears that the poorer growth obtained with Argentine RSM than with Polish RSM (Clandinin et al., 1959) might have been partly attributable to the lower lysine content of the rations containing Argentine RSM. The possibility that lysine was limiting in RSM was also reported by Blakely and Anderson (1948), Kratzer et al. (1954) and Klain et al. (1956). For example, Kratzer et al. (1954) found that additional lysine was needed for optimum growth of chicks and optimum growth and feather pigmentation of turkey poults fed practical rations containing rapeseed meal. Clandinin et al. (1959) compared the effect of three temperature conditions on protein quality of expeller RSMs. Chicks that received RSM having undergone the lowest temperature processing (cooker, 220°F, 30 min; conditioner, 240°F, 5 min) gained more weight (365 gm vs 324 gm and 275 gm) than chicks fed RSM processed at higher temperature conditions (234°F-264°F and 250°F-280°F, cooker/conditioner, respectively). Also of interest was the observation that even the lowest temperature conditions resulted in appreciable reduction in the amount of lysine. Lysine content of the seed before oil extraction was 6.42% whereas the lysine content of the meals processed at low, medium and high temperatures was 5.69, 4.86 and 4.12%, respectively. Although the highest processing temperature was higher

than "normal" these observations indicate that the lysine content of RSM can be reduced markedly by heat processing. However a recent cooperative study (Giovanetti and Bell, 1971) carried out in Canada indicated that method of processing (expeller, prepress-solvent and solvent processes) had no effect on the amino acid composition of the meals; lysine contents of the different samples of RSM were essentially the same.

Thus it appears that there has been a marked improvement in the processing of rapeseed meal during the past 10 to 15 years. Nevertheless the possibility of destruction of amino acids, particularly lysine, still remains with the expeller RSM, if processing temperature is not carefully controlled.

2.2 Availability of amino acids in different protein sources

As described earlier rapeseed meal appears to be more poorly digested than other types of protein such as casein, soybean protein, fish meal, zein and most cereal proteins except oats (Drouliscos and Bowland, 1969; Oliver et al., 1970; Tao et al., 1971; Flipot et al., 1971; Cho and Bayley, 1970; Carlson and Bayley, 1970; May and Bell, 1971). Amino acid availability data obtained by the fecal analysis method (Kuiken and Lyman, 1948; Kuiken, 1952; Olsen et al., 1968; De Muelenaere and Feldman, 1960; Cho and Bayley, 1970; Carlson and Bayley, 1970; Giovanetti et al., 1970; Flipot et al., 1971; Tao et al., 1971; Sauer, 1972; Nielsen, 1968) indicate that some amino acids are more available than others although amino acid availability depends on the type of protein being studied. Kuiken and Lyman (1948) found that all essential amino acids in roast beef were completely available to the rat whereas wide variations occurred in the availability

of the essential amino acids in cottonseed flour. Lysine in cottonseed meal was only 65% available. The corresponding value for arginine in the same sample was 93%. The availabilities of arginine, histidine, phenylalanine, leucine and isoleucine were greater than that of the other essential amino acids. Similarly Olsen et al. (1968) reported that there was considerable variation among amino acids in their percentage absorption from wheat by-products. The absorption was highest for cystine, glutamic acid, histidine and arginine in all the products tested whereas the lowest percentage absorption was found for threonine, lysine, alanine, glycine, aspartic acid, methionine and isoleucine. Nielsen (1968) found that the apparent digestibilities of methionine, threonine and alanine were significantly lower than those of other amino acids in a diet containing barley, soybean meal, meat and bone meal and skim-milk protein. The true digestibilities of glutamic acid, proline, serine, lysine, phenylalanine, arginine and histidine in soybean meal for the young pig were found to be higher than those of alanine, threonine, methionine, isoleucine and glycine (Carlson and Bayley, 1970; Sauer,1972). Giovanetti et al. (1970) and Sauer (1972) also reported lower availabilities for lysine, alanine, threonine and methionine in triticale, wheat and barley, in contrast to the high digestibilities of proline glutamic acid, arginine, histidine and phenylalanine. Cho and Bayley (1970) found that apparent digestibilities of most amino acids in prepress-solvent RSM were significantly lower than those of soybean meal, especially for isoleucine, proline, serine, aspartic acid and phenylalanine. However, the apparent digestibility of methionine was significantly higher for rapeseed meal than for soybean meal. Several amino acids followed

a similar pattern of absorption with both proteins. Glutamic acid, proline and lysine were well utilized whereas threonine, valine and isoleucine were poorly absorbed. Similar observations were reported (Tao et al., 1971) for rapeseed meal with colostomized chicks. Apparent digestibility coefficients (ADC) for lysine, methionine, arginine, glutamic acid and proline ranged from 64 to 72% whereas ADC for threonine, valine, isoleucine, aspartic acid, serine, tyrosine and phenylalanine ranged from 44 to 57%.

Thus in general proline, arginine and histidine are well absorbed regardless of protein sources whereas the digestibility of alanine and threonine generally tends to be low. However, the observed digestibilities of individual amino acids in different protein sources may be confounded by their relative content in the protein. A rather poor digestibility of lysine has been reported for barley and wheat, which are low in lysine. Lysine availability has been found to be comparable to that of the other amino acids in soybean meal whereas methionine, which is the most limiting amino acid in the soybean meal, is the amino acid with the poorest digestibility.

2.3 Factors affecting amino acid availability in protein

2.3.1 Specificity of the enzyme hydrolysis

The absorption of protein requires hydrolysis to amino acids (Van Slyke and Meyer, 1912; 1913; Abel et al., 1914) except in the fetus and newborn (Wilson, 1962). Enzymatic cleavage of protein is therefore very important to amino acid availability. Three sources of proteolytic enzymes contribute to the hydrolysis of proteins; namely,

gastric, pancreatic and intestinal secretions.

The main gastric secretion is pepsin, an endopeptidase. Pepsin is a rather non-specific enzyme although it preferentially attacks the peptides bonds adjacent to leucine and aromatic amino acids such as tryptophan, phenylalanine and tyrosine. There may also be some attack adjacent to cysteine and cystine (Mahler and Cordes, 1968).

Pancreatic secretions contain the proteolytic enzymes trypsin, chymotrypsin, elastase and carboxypeptidases A and B. These are highly specific proteolytic enzymes (except elastase) and are secreted by the pancreas in their "zymogen" form which is inactive. Trypsin catalyzes the hydrolysis of peptides bonds whose carbonyl function is donated by a basic amino acid, usually arginine and lysine. However, trypsin does not hydrolyze peptides bonds if the α-amino group of lysine or arginine is free or if lysine is followed by proline in the peptide chain (Dixon et al., 1958; Hirs et al., 1959). The action of chymotrypsin is not as specific as that of trypsin although chymotrypsin generally hydrolyzes proteins at the carboxyl group of aromatic amino acids such as phenylalanine, tyrosine and tryptophan, providing they are not terminal amino acid residues. However, the enzyme is also capable of hydrolysis peptide linkages adjacent to leucine, valine, glutamic acid and histidine (Dixon et al., 1958; Hirs et al., 1956; Leonis et al., 1959). Carboxypeptidases A and B, which are exopeptidases, degrade polypeptides in a sequential fashion beginning at the C terminus. The specificity of carboxypeptidase "A" is primarily for C-terminal aromatic residues although it can act on bonds adjacent to other amino acids with the order of specificity being methionine> isoleucine> alanine>

glycine. Carboxypeptidase "B" acts only on peptides containing arginine and lysine in the C-terminal position (Neurath, 1960). Another endopeptidase, elastase, which has been identified in the pancreatic juice of the pig (Lewis et al., 1956), rat and guinea pig (Cohen et al., 1958), has a broad specificity and can attack a wide variety of peptide bonds involving neutral amino acids having aliphatic side-chains (Naughton and Sanger, 1961).

The intestinal mucosa also contains a number of proteolytic enzymes namely polypeptidases, prolidases, tripeptidases and dipeptidases. These enzymes complement the action of gastric and pancreatic enzymes by affecting the cleavage of peptides resulting from the partial breakdown of dietary proteins. One important enzyme responsible for most polypeptidase activity is leucine amino peptidase (Spackman et al., 1955). This enzyme is an exopeptidase and degrades polypeptide chains sequentially from the N-terminus. As the name of the enzyme implies hydrolysis is most rapid in the case of N-terminal leucine but this enzyme also attacks peptides where the N-terminal amino acid is hydrophobic (Smith and Hill, 1960). On the other hand the presence of a polar side chain on N-terminal amino acid greatly reduces the rate of hydrolysis. Dipeptidases, which split specific dipeptides, e.g. alanine-glycine, glycine-glycine, proline-glycine, have been described (Hill, 1965). Peptides containing these amino acids may be split on the mucosal surface of the intestinal wall (Ugolev et al., 1964) or in the mucosa cells (Newey and Smyth, 1959; Wiggans and Johnston, 1959).

Although the specificity of action of the proteolytic enzymes has been well established, its importance on the in vivo availability of

various amino acids in proteins is not fully understood. It is possible that the lower digestibility of some proteins, e.g. rapeseed meal, zein, is due to resistance of specific peptide bonds to hydrolysis by proteolytic enzymes because of their particular position in the polypeptide chain. More information is needed concerning the relationships among specificity of enzyme attack, protein configuration and the availability of amino acids.

2.3.2 Response of digestive enzymes to dietary proteins

Release of amino acids from proteins depends, as described in the previous section, on the hydrolysis of protein by proteolytic enzymes. In addition to the specificity of the proteolytic enzymes there is some evidence that rate of synthesis and activity of these enzymes in the intestinal lumen may be related to the source of dietary protein.

The importance of pancreatic secretions in digestion of protein is well illustrated by the studies with raw soybean meal. Early workers thought that the poor utilization of raw soybean meal was due to the impaired release of amino acids particularly methionine (Bowman, 1944; Riesen et al., 1947; Melnick et al., 1946) because fecal excretion of nitrogen and sulfur amino acids was increased when raw soybean was fed (Evans et al., 1947). However, the hypothesis that protein digestion was depressed in animals fed raw soybean was challenged by several researchers (Chernick et al., 1948; Lyman and Lepkowsky, 1957; Lyman, 1957; Lepkowsky et al., 1959; Haines and Lyman, 1961) who reported a marked increase in pancreatic secretion and in intestinal tryptic activity in rats and chickens fed raw soybeans. Intestinal trypsin activity which was low immediately before eating increased steadily after a meal of raw

soybean to a level about 3 times that in animals fed heated meal. These researchers concluded that excessive stimulation of the pancreas occurred in response to the ingestion of raw soybean and that the proteins contained in these secretions were ultimately lost by the animal thereby producing growth depression. This hypothesis was supported by the beneficial effects of amino acid supplements, particularly methionine, on growth of the animal (Fisher and Johnson, 1958; Borchers, 1961) and also by histological studies of the pancreas (Booth et al., 1960) which suggested that prolonged feeding of raw soybean meal induced pancreatic hypertrophy.

Other studies suggest that dietary proteins which do not contain trypsin inhibitors also influence synthesis, secretion and inactivation of digestive enzymes, especially trypsin and chymotrypsin. For example, Abdeljlil and Desnuelle (1964) and Howard and Yudkin (1963) found that the pancreas "adapts" to a high level of casein in the diet by increasing the synthesis of proteases and decreasing the synthesis of amylase. Wang and Grossman (1951) reported that the presence of amino acids and peptones in the upper intestine caused an increase in the enzymatic output of the pancreas. They also found that lysine, tryptophan and phenylalanine were the only amino acids, of those tested, which stimulated pancreatic enzyme secretion as effectively as peptones. Snook and Meyer (1964a, 1964b) and Snook (1965) found that source of dietary protein had an influence on the secretion and the activity of proteolytic enzymes. The content of chymotrypsinogen and trypsinogen in the pancreas was lower on a nitrogen-free diet and higher on a whole egg protein diet than the levels of "zymogen" observed with casein. Furthermore, hydrolyzed casein and

hydrolyzed egg protein was more effective than the corresponding intact protein in the induction of "zymogen" synthesis in the pancreas when the activated pancreatic gland was assayed 2.5 hr after the initiation of a 1 hr feeding period. The increased synthesis of zymogen in the pancreas in response to protein was accompanied by an increase in endogenous nitrogen as well as total trypsin and chymotrypsin activity in digesta from the small intestine. Gelatin and a mixture of amino acids increased trypsin and chymotrypsin activity to an even greater extent than casein, hydrolyzed casein, zein and a nitrogen-free diet. Snook (1965) concluded that differences in the secretory response elicited by the different diets must be principally the result of variations in the proteins, peptides and pattern of amino acids in the intestine with the response being mediated through neural and hormonal mechanisms.

In addition to their effect on the absolute amount of enzymes secreted into the intestinal lumen different types of proteins affect the proteolytic activity through their action on the digestive enzymes. The protective effect of exogenous proteins against inactivation of the proteolytic enzymes is particularly important with less stable enzymes such as chymotrypsin. Snook and Meyer (1964b) found that the chromatographic properties of trypsin and chymotrypsin from contents of the small intestine were altered to a greater degree when rats were fed a protein-free diet than when they were fed diets containing 15% protein. The relative activity of chymotrypsin to trypsin (Ch/Tr ratio) was considerably lower in the gut of rats fed an amino acid mixture or a nitrogen-free diet than that of rats fed bovine serum albumin and whole egg protein. It was suggested by Snook (1965) that the Ch/Tr activity

ratio in the small intestine was related to the relative ability of proteins to retard the inactivation of pancreatic enzymes and also to the rates at which peptides are released from the protein within the stomach and emptied into the small intestine. Thus it appears possible that certain types of proteins may be more completely digested because they prevent the rapid breakdown of proteolytic enzymes, particularly chymotrypsin.

The fact that, in many cases, dietary proteins modify synthesis, activation and inactivation of pancreatic proteolytic enzymes suggests that they may in turn affect the rate of release of amino acids from proteins and the amount of metabolic fecal nitrogen. However, more work is needed on the response of digestive enzyme secretions to different types of protein. Evidence that the secretory response of the pancreas was much less on a protein-free diet than on a diet containing protein suggests that metabolic fecal nitrogen may be underestimated by a protein-free diet resulting, in turn, in a corresponding underestimation of true amino acid availability.

2.3.3 Rate of amino acid absorption

It is generally accepted that digestion is the rate limiting step in the absorption of protein. However, the frequent lack of correlation between plasma amino acid levels and the quantities of these amino acids ingested raises the question of whether rate of release of amino acids is the only factor affecting the availability of amino acids. Factors such as molecular weight, affinity for different

transport systems, competition for absorption sites and antagonisms among amino acids have been reported to affect rate of absorption of individual amino acids (Kratzer, 1944; Wiseman, 1955, 1956; Delhumeau et al., 1962; Orten, 1963; Adibi et al., 1967).

However, whether these factors apply to absorption of amino acids released from proteins has been challenged by several researchers (Nasset, 1957; Bergen and Purser, 1968; Coulson and Hernandes, 1970; Nixon and Mawer , 1970a, 1970b). Nasset (1957) concluded that the relative rates of absorption of different amino acids were similar for specific dietary protein sources because amino acid patterns remained constant in digesta protein from different levels of the intestinal tract. Similarly, the relatively constant amino acid composition of the gut content was interpreted by Bergen and Purser (1968) and Nixon and Mawer (1970a) as an indication that no differential absorption occurred among amino acids from food digesta in rat and man. Nixon and Mawer (1970a) reported that, in man fed either milk protein or gelatin, similar molar ratios for most amino acids were found in digesta samples collected from 81 cm to 233 cm from the nose. The rates of absorption of the amino acids for both proteins were proportional to the concentration of these amino acids in these proteins except for arginine, alanine, proline and glycine. Glutamic acid and methionine were absorbed at a similar rate although it has been reported in experiments with amino acid mixtures (Delhumeau et al., 1962; Orten, 1963; Adibi et al., 1967) that glutamic acid was poorly absorbed relatively to the rapid absorption of methionine. The relative concentration of glutamic acid and methionine remained constant throughout the gut and did not increase (in the case of glutamic acid) or decrease (in the case of methionine) appreciably.

Thus there does not appear to be any relationship between the rate of absorption of individual amino acids from intact proteins with that from an amino acid mixture.

Coulson and Hernandez (1970) compared the rate of absorption of amino acids liberated from fish proteins with the rate of absorption of single amino acids in the cayman. One of the reasons these researchers selected the cayman relates to this species' ability to consume enormous amounts of food relative to their body weight and to the fact that the slow rate of digestion in this animal allows more accurate determination of kinetics of amino acid absorption. Coulson and Hernandez (1970) found that all amino acids were released from food protein at similar rates. Absorption of amino acids from fish protein was rapid compared with the rate of absorption of single amino acids and only a small proportion of free amino acids accumulated in the intestine of the cayman fed the fish diet. There was also evidence that absorption of amino acids could have been more rapid if free amino acids had been more rapidly released by the digestive enzymes. Coulson and Hernandez (1970) concluded that the rate of absorption of amino acids from a protein digest was unrelated to the rate of absorption of single amino acids.

Thus the characteristic rate of absorption of individual amino acids does not appear to account for difference in the rate of absorption of amino acids from dietary proteins. The constant amino acid composition of digesta at different sites along the gut and evidence of a rapid absorption of all amino acids released during the digestion of protein suggest that the rate limiting factor in the digestion and absorption of a protein is rate of amino acid release from the protein and not the rate of absorption of individual amino acids. It is unlikely that

competition occurs among amino acids when absorption sites are not saturated with amino acids. Rate of absorption therefore will be proportional to the concentration of amino acids in the diet and their rate of release during digestion.

2.3.4 Effect of heat processing on the nutritional value of protein

The preparation of a food by commercial processing or culinary practice has been found to modify the utilization of protein; both its digestion and metabolism. In general, heating does not improve the nutritive value of proteins of animal origin, rather the method and the degree of heating will determine whether there will be a loss of nutritive value. With certain plant protein sources, however, moderate heat has been shown to enhance their nutritive value. Since oil seed proteins are subjected, either purposely or accidently, to variable amounts of heat during processing it may be worthwhile to review the effect of heat on protein utilization.

A. Beneficial effect of heat treatment

Although severe heating during processing has been demonstrated to generally decrease the nutritive value of protein, heating has been found to aid the utilization of some protein sources. The beneficial effect of heating proteins that contain a trypsin inhibitor such as raw soybean (Ham and Sandstedt, 1944; Bowman, 1944; Melnick et al.,1946; Riesen et al., 1947), lentils, kidney beans and pinto beans (Griswold, 1951) has been well established in feeding trials with rats (Osborne and Mendel, 1917; Melnick et al., 1946; De Muelenaere, 1964), pigs (Robison, 1930; Vestal and Shrewsbury, 1932) and chickens (Wilgus et al., 1936; Hayward et al.,1936b; Evans et al., 1947). This beneficial effect of heat probably explains why Melnick et al. (1946) found that the in vitro digestion of expeller soybean meal which is subjected to a certain

amount of heat during compression of seeds was greater than that of solvent processed soybean meal. However both meals required autoclaving for 10 to 30 minutes in order to adequately inactivate the trypsin inhibitor (Melnick et al., 1946; Evans et al., 1947). Another example of the favorable effect of processing on protein utilization was the observation by Mitchell et al. (1949) that the digestibility of expeller linseed meal was significantly ($P < 0.02$) higher than that of solvent processed linseed meal.

Improvement in nutritive value of raw soybean as a result of heat treatment appears to be mainly the result of an improvement in metabolic utilization of the protein (Hayward et al., 1936a,b) although some studies (Melnick et al., 1946; Evans et al., 1947; Kwong et al., 1962; De Muelenaere, 1964) indicate that mild heat treatment increased appreciably (4-13%) the digestibility of raw soybean. De Muelenaere (1964), for example, reported that the true digestibility of raw, properly heated and overheated soybean meal with rats was 82.9, 89.7 and 84.0 percent, respectively. As discussed earlier, the improved utilization of properly heated soybean meal may be attributable to a decreased loss of metabolic nitrogen (Lepkovsky et al., 1959; Booth et al., 1960). De Muelenaere (1964) also concluded that the lower protein digestibility and availability of amino acids in raw soybean meal might be attributable to greater excretion of metabolic nitrogen and impaired amino acid absorption rather than poorer digestion of this meal. The question of whether abnormal excretion of particular amino acids, e.g. methionine, also is involved in the poor utilization of raw soybean has been studied by Melnick et al. (1946) and De Muelenaere (1964). They indicated that the excretion of methionine was greater

than that of protein with raw soybean and that the lower relative availability of methionine might be the limiting factor in the utilization of this protein. This conclusion also was confirmed by the fact that nutritional quality of the raw product could be improved by the addition of certain amino acids, particularly methionine (Hayward and Hafner, 1941; Fisher and Johnson, 1958; Borchers, 1961; Barnes et al., 1962). Yet addition of amino acids to raw soybean did not appear to be as effective as heat treatment in the improvement of the nutritional quality of soybean meal (Saxena et al., 1962).

Regardless of the mechanism involved, heat treatment such as mild autoclaving or cooking play an important role in improving the nutritive value of protein sources such as soybean meal and perhaps linseed meal. Similarly, heat treatment appears to improve the utilization of rapeseed meal as a result of the inactivation of the myrosinase (Mustakas et al., 1962; Belzile et al., 1963; Woodly et al., 1972), the enzyme responsible for the release of the goitrogenic substances. However, this effect of heat on rapeseed meal is probably due to an improvement in food intake rather than an effect on digestibility and metabolic utilization of the protein. Thus with the exception of certain leguminous protein sources the beneficial effect of heat does not appear to be due to improved utilization of the protein per se.

B. Detrimental effect of heat treatment

The detrimental effect of heat treatment on protein quality and digestibility has been demonstrated for a variety of protein sources, such as milk protein (Fairbank and Mitchell, 1935; Hankes et al., 1948; Henry et al., 1948; Henry and Kon, 1950; Hodson and Krueger, 1947), cereals (Morgan, 1931; Stewart et al., 1943; Mitchell et al., 1949),

meat (Mayfield and Hedrick, 1949; Beuk et al., 1948; Ford and Salter, 1966), fish meals and oilseed meals (Evans et al., 1947; Mitchell et al., 1949; Evans and Butts, 1949; Kuiken, 1952; Clandinin, 1949; Morrisson et al., 1953).

Experimental evidence suggests that three mechanisms are involved in the decreased utilization of proteins as a result of heat damage. One mechanism involves an impairment of digestion whereas another involves a decrease in the metabolic utilization of the amino acids and the third involves irreversible chemical destruction. The Maillard reaction, a reaction between sugar aldehyde and free amino groups in proteins, particularly the ε-amino group of lysine, results either in impaired digestion or decreased metabolic utilization of the amino acid. Under mild processing conditions (storage, short period of autoclaving) the Maillard reaction between amino groups and reducing sugars does not cause destruction of amino acids although their availability is impaired. Under more severe heat treatment this type of reaction leads to polymerization of carbohydrate-lysine compounds yielding brownish-colored products. Simultaneously, there is an extensive loss or destruction of other amino acids. The presence of reducing sugar or carbohydrates is not absolutely necessary for change to take place in protein quality. Heat damage also occurs with pure protein preparations, meat and fish products which are essentially free of carbohydrates (Carpenter et al., 1963). This type of heat damage also is accompanied by a loss of reactivity of the ε-amino group of lysine. It has been hypothesized (Bjarnason and Carpenter, 1970) that these changes are due to the formation of an unnatural "amide" bond between the ε-NH_2 group of lysine and free carboxyl group of aspartic

and glutamic acids although a recent report from the same laboratory casts doubt on the hypothesis (Waibel and Carpenter, 1972).

2.3.5 Effect of heat on *in vivo* and *in vitro* digestion of proteins

Heat treatment has been found to decrease the digestibility of proteins (Fairbank and Mitchell, 1935; Evans *et al.*, 1947; Mitchell *et al.*, 1949; Ford and Salter, 1966). Fairbank and Mitchell (1935) reported that roller drying decreased the true digestibility of milk protein from 94.8 to 81.4% and the biological value from 89.8 to 69.8%. Studies by Clandinin (1949) with fish meal, Morrisson *et al.* (1953) with sunflower meal and Mitchell *et al.* (1949) with several protein sources indicated that commercial processing can decrease the nutritive value of food protein. Mitchell *et al.* (1949) found that the biological values of peanut meal, sunflower seed meal and cottonseed flour were significantly reduced by expeller processing or autoclaving. The true digestibility of expeller processed cottonseed flour was 5.1% less than that of the solvent processed meal whereas method of processing had no effect on the digestibility of peanut protein. They also found that autoclaving sunflower seed meal and flaking corn resulted in a decrease in digestibility of 2.5 and 14.2%, respectively, but roasting had no effect on the digestibility of beef. Kuiken (1952) reported lower digestibility for expeller processed cottonseed meal (77-87%) compared to solvent processed meal (92%) whereas Saben *et al.* (1971a) found no differences in digestibility among expeller, pre-press solvent and solvent processed rapeseed meals. Although it is difficult to compare results by different authors it appears that the adverse effect of heat on the digestibility and biological value of a protein may depend on the source of the protein.

It appears that the lower digestibility of heated food is due to molecular changes which render the protein particularly resistant to proteolytic digestion. The greater susceptibility of cottonseed meal to heat damage may be related to the fact that gossypol combines with the free amino group of lysine and thereby inhibits digestion. *In vitro* digestion experiments have shown that heating of cottonseed meal in the presence of gossypol leaves the protein resistant to tryptic and peptic digestion (Baliga *et al.*, 1959). Studies with soybean protein (Evans and McGinnis, 1948; Riesen *et al.*, 1947; Evans and Butts, 1949), casein (Hankes *et al.*, 1948) and fish protein (Ford, 1965; Ford and Salter, 1966) indicate that the availability of lysine, methionine, arginine, glutamic acid, cystine and tryptophan are particularly affected by heat. Riesen *et al.* (1947) found that 9.5% of the lysine, 32.6% of the methionine and 43.3% of the tryptophan were released from overheated soybean meal (autoclaved 4 hrs at 15 lbs pressure) during a 5 day *in vitro* pancreatin and erepsin digestion. In contrast the percent of lysine, methionine and tryptophan released from properly heated soybean meal (4 min at 15 lbs pressure) was 32.4, 56.2 and 60.0%, respectively. Similarly, Hankes *et al.* (1948) found that autoclaving casein for 20 hours resulted in a marked decrease in the rate of liberation (by pepsin pancreatin and erepsin) of lysine, threonine, aspartic acid (54-55% were released), methionine (65%) and glutamic acid (78%). The extent of enzymatic release of amino acids and their pattern of digestion appears to be dependent upon the carbohydrate content of the protein. Evans and Butts (1949) reported that autoclaving soybean protein with sucrose (20% W/W) not only considerably reduced the overall rate of *in vitro*

digestion but also the relative proportion of amino acid released. Thus the extent of release of cystine, lysine, arginine, glutamic acid, histidine, methionine and aspartic acid was 14, 16, 45, 50, 58, 59 and 66 percent, respectively, in soybean protein heated with sugar, whereas it was 86, 70, 92, 76, 84, 94 and 73 percent, respectively in soybean protein heated in the absence of sugar. The enzymatic release of other amino acids such as phenylalanine, threonine, leucine, isoleucine and valine were not markedly affected by heat treatment either in the presence or absence of sucrose. As a result of these observations, Evans and Butts (1949) concluded that heat treatment affected the enzymatic release of those amino acids with free amino or carboxyl groups or with other active groups such as the sulfur of cystine or methionine and the imidazole group of histidine. They also suggested that aspartic and glutamic acid from proteins devoid of carbohydrates were not readily released because the free carboxyl of these amino acids reacted with the ε-amino group of lysine to give linkages resistant to enzymes hydrolysis. This postulation is supported by the sophisticated studies of Ford (1965) and Ford and Salter (1966) who found relatively poor release of lysine, cystine, glutamic acid, aspartic acid and methionine in digesta of heated fish meals. However, as mentioned previously, Waibel and Carpenter (1972) question this hypothesis.

Heating or storing proteins in presence of sugar has also been found to decrease *in vivo* digestibility (Henry and Kon, 1950; Shroeder *et al.*, 1961; Boctor and Harper, 1968; Valle-Riesta and Barnes, 1970). Valle-Riesta and Barnes (1970) found that the digestibility of ovalbumin, autoclaved with 16% glucose, was much lower (39%) than that of the same

protein (64%) autoclaved under the same conditions, but in absence of glucose. These results coincide with the findings that heat damage in the presence of sugar severely depresses *in vitro* digestion of soybean protein (Evans and Butts, 1949).

Furthermore, digestibility trials with heated proteins indicate that heat damage decreases the availability of most amino acids and not just amino acids that specifically undergo the Maillard type reaction (e.g. lysine, arginine). The nutritional availability of lysine (Valle-Riesta and Barnes, 1970) was reduced to the same degree as total dietary nitrogen in heat damaged ovalbumin. Boctor and Harper (1968) also found that heat treatment of egg albumin resulted in considerable fecal excretion of all essential amino acids and that the digestibility of lysine was similar to the other essential amino acids. Miller *et al.* (1965) and Varnish and Carpenter (1970) reported that the nutritional availability of lysine, methionine and tryptophan were reduced to a similar degree in severely heated animal proteins. Similarly, the digestibility of methionine, leucine, valine and threonine was reduced to a similar extent to that of lysine in expeller cottonseed meal as compared to solvent processed meal (Kuiken, 1952).

Although the digestibility of lysine in heat damaged protein is similar to that of other amino acids, *in vitro* and *in vivo* studies indicate that the "biological availability" of lysine may be severely impaired by heat treatment (Gutneck *et al.*, 1952; Gupta *et al.*, 1957; Ford and Salter, 1966; Boctor and Harper, 1968). Three possibilities have been suggested to explain these apparent discrepancies: 1) digestive release and absorption of the lysine-carbohydrate complex occurs but the

lysine-CHO complex is not metabolized by the body (Bjarnason and Carpenter, 1969; Valle-Riesta and Barnes, 1970; Mauron, 1970): 2) there is an impaired absorption of the lysine-carbohydrate complex with the lysine being broken down by the intestinal microorganisms and not recovered in the feces; and 3) that the slow rate of release of lysine compared to other amino acids, rather than digestibility of lysine (Pader et al., 1948; Ford, 1965; Ford and Salter, 1966) may increase the requirement for lysine in order to ensure the efficient utilization of the other amino acids. More research needs to be done to ascertain which, if any, of these possibilities explain the observation that lysine is poorly available in heat damaged proteins.

There is no doubt that excessive heat during processing can have a profound effect on the availability of amino acids in food. The adverse effect of heat depends not only on the severity of heat treatment but on amount and type of carbohydrate in the foodstuff. However accurate determination of the amount of amino acids "available" to the animal fed heated proteins remains a problem with the possibility that factors other than digestibility are important in the efficient utilization of amino acids from a protein.

2.4 Factors affecting amino acid pattern in digesta and feces

2.4.1 Introduction

Several groups (Payne et al., 1968; Giovanetti et al., 1970; Sauer , 1972; Cho and Bayley, 1972) have recently suggested that amino acid pattern in digesta and feces may be useful in the determination of amino acid availabilities. However, the presence of a variable amount of endogenous protein during the digestive processes mask to a

certain extent the amino acid composition of dietary proteins as well as the proportion of non-absorbed amino acids. Other factors such as fiber, feed intake, protein level and microflora also have been found to affect the amount and composition of fecal nitrogen. Some knowledge of the relative importance of each of these factors is necessary for the interpretation of the data on the availability of amino acids in different protein sources.

2.4.2 Dilution by endogenous protein

The primary sources of endogenous protein in digesta and feces are the gastrointestinal secretions (60-70% of total) such as saliva, gastric juice, pancreatic and intestinal juices and protein from the desquamation of the epithelial lining of the gut (30-40% of the total). The magnitude of endogenous nitrogen secretion into the gastro-intestinal tract is particularly important. Dreisbach and Nasset (1954) observed that the amount of protein recovered in digesta from the small intestine of rats fed an 18% casein diet was equal to or greater than the amount of protein ingested. Meyer et al. (1959) using nitrogen-lignin ratios calculated that the nitrogen content of digesta increased 175% on passing from the abomasum through the small intestine of sheep. Twombly and Meyer (1961) calculated that the amount of endogenous protein secretion in the gastrointestinal tract of the rat was equivalent to the amount of nitrogen consumed with a 10% protein diet. They also reported that only about 10% of all endogenous protein secreted in the gastrointestinal tract appeared in the feces of rats fed whole egg protein. A considerable fraction (90%) of the metabolic nitrogen is therefore reabsorbed. Furthermore, Nasset (1965) estimated that 64-263 gm of protein were

secreted daily by the human with intestinal juice accounting for 40-200 gm and pancreatic juice 12-70 gm. Since the entire mucosa of the small intestine is replaced every 1-3 days, desquamated cells could account for 77-91 gm of protein per day in an adult man. Thus the secretion of endogenous protein in the small intestine of man appears to be about equivalent to the protein intake. Other observations by Nasset and associates (1955, 1957, 1961, 1963; Ganapathy and Nasset, 1962; Olmstead et al., 1966) suggested that the blending of endogenous proteins with various dietary proteins tends to provide a relatively constant amino acid mixture for absorption from the small intestine. They found the same relative amino acid pattern in the digesta from the small intestine of dogs at 1.5 hr after being fed albumin, zein and a nitrogen-free diet (Nasset, 1955). Although zein is almost devoided of lysine and tryptophan these amino acids accounted for 3.0 and 0.5 percent, respectively, of the total amino acids in the digesta from the small intestine of dogs fed these proteins. An accurate estimate of the magnitude of endogenous dilution of dietary proteins using ^{14}C labeled casein indicated a 9-fold dilution for dogs and 7-fold for rats. Nasset (1965) concluded that the presence of a large amount of endogenous nitrogen in the lumen acts as a homeostasis device to prevent wide fluctuation in the amino acid mixture available for absorption.

On the other hand studies other than those of Nasset and co-workers indicate a somewhat lower dilution of dietary proteins by endogenous proteins in the intestine. Experiment by Ochoa-Solano and Gitler (1968) showed a three-fold dilution of exogenous proteins by endogenous proteins in the intestine of rats fed ^{75}Se-selenomethionine and ^{35}S-methionine

labeled ovalbumin. Similarly Cho and Bayley (1972) observed a two fold dilution in the duodenum of pigs fed either rapeseed meal or soybean meal. Crompton and Nesheim (1969) reported that the extent of dilution of dietary proteins, in the intestine of duck, was 50% of the exogenous intake. Nixon and Mawer (1970a) calculated that the amount of endogenous protein secreted in man in response to the test diets ranged from 13% on casein to 53% on gelatin when the endogenous protein was expressed as percent of the protein intake. The lower dilution of dietary protein by metabolic nitrogen observed by Cho and Bayley (1972), Crompton and Nesheim (1969), Nixon and Mawer (1970a) was reflected by the similarity between amino acid pattern in digesta from the upper jejunum and that of dietary protein. However, there was a marked difference between the amino acid pattern of the diet and that of digesta from the lower region of the intestinal tract. These observations suggest that digestion of dietary proteins occurs before that of endogenous proteins probably because the latter are more resistant to enzymatic hydrolysis. A similar suggestion also was presented by Twombly and Meyer (1961). They found that there were two peaks in the nitrogen content of the small intestine of rats; one at 1 hr after feeding and the second at 8 hours. The first peak was thought to be due to the accumulation of dietary protein in the gut and the second to the accumulation of slowly digested endogenous secretions. Exogenous proteins appear to be more rapidly digested than endogenous protein and the accumulation of the latter may explain the relatively constant amino acid pattern observed by Nasset and associates in their studies with dogs and rats. Dilution of dietary proteins by endogenous proteins will be to some extent related to the rate of absorption of dietary proteins.

Bergen and Purser (1968) found that the ratios of exogenous and endogenous amino acids in the upper jejunum of rats were 1:1 with casein, 1:1 with bacterial protein and 1:4 with protozoal protein. In the ileal content, the dilution ratio was 1:10 for casein compared to 1:2 with bacterial and protozoal protein. Since the true digestibility of casein, protozoal protein and bacterial protein were 97, 87, and 75%, respectively, the contribution of endogenous protein to the total nitrogen in digesta and feces was particularly pronounced with well digested proteins such as casein. Most of the studies by Nasset and co-workers were conducted using highly digestible protein, i.e. casein, meat, egg protein, which would be rapidly absorbed and thus the relative proportion of endogenous protein to dietary proteins would be relatively high.

In summary, results obtained from the experimental work discussed appear to indicate a 1-2 fold dilution of exogenous protein by endogenous protein which is considerably less than that suggested by Nasset. Absorption of endogenous protein tends to occur after that of dietary proteins at least with very digestible proteins. However , accurate determination of rate of absorption of individual amino acids is rather difficult because there is no way to distinguish between amino acids of endogenous origin from those of exogenous proteins. More work needs to be carried out on the factors affecting the secretion of endogenous protein.

2.4.3 Effect of various factors on metabolic fecal nitrogen excretion

A. Effect of fiber

The influence of dietary fiber on metabolic nitrogen excretion has

been recognized since the turn of the century. Mendel and Fine (1912) observed an augmentation in metabolic fecal nitrogen excretion when the non-digestible material in the diet was high. They suggested that the nitrogen-free diet be adjusted to contain the same amount of indigestible material as the test diets in true digestibility determinations. Similarly, Mitchell (1924), Schneider (1934) and Meyer (1956) found that it was important to adjust the fiber content or cellulose level of the diet in determination of metabolic fecal nitrogen (M.F.N.)

The importance of the fiber level on the digestibility of protein was extensively studied by Behm (1954), Whiting and Bezeau (1957a, 1957b) and Zelter and Charlet-Lery (1961). Behm (1954) reported that fecal nitrogen losses were 0.099, 0.129 and 0.199 gm per 100 gm dry matter for pigs fed N-free rations containing 5,10 and 20% cellulose, respectively. Whiting and Bezeau (1957a) demonstrated that the increase in M.F.N., as the level of cellulose increased in the diets of pigs, caused a significant decrease in the apparent and true digestibility of protein. For example, the average apparent digestibility of an 18% protein diet was 87, 85 and 83% when the diet contained 5, 10 and 15% of cellulose, respectively, whereas true digestibility coefficients were 91, 89 and 88%. True digestibility coefficients were determined by correcting for M.F.N. at each level of cellulose. Fiber in the ration seemed to interfere with absorption of the dietary protein as well as markedly increasing the amount of M.F.N. They found that the relationship between M.F.N. and level of cellulose and protein was linear and that these relationships could be expressed by the following regression equations:

Level of fiber 5% $Y = 0.102+0.017X$

Level of fiber 10% $Y = 0.126+0.020X$

Level of fiber 15% $Y = 0.125+0.023X$

where Y is fecal N excretion per 100 gm D.M. intake for pigs weighing approximately 37 kg and X is the percentage of protein in the air-dry ration over the range 0.3 to 22% protein. It can be calculated from these equations that excretion of fecal nitrogen at a protein level of 18%, will be increased by 16% when the percentage of cellulose of the diet is increased from 5 to 10%.

Whiting and Bezeau (1957b) also found that the type of fiber as well as amount significantly affected the fecal N output of the pig whether expressed on the basis of dry matter (D.M.) intake or fecal D.M. output. Values of 0.13, 0.09 and 0.07 gm fecal N/100 gm of D.M. intake were obtained for Solka-Floc (wood cellulose), oat hulls and methocel (methylcellulose), respectively, when the diet contained 14% fiber and 0.3 protein. The corresponding fecal N outputs/100 gm D.M. excretion were 0.68, 0.59 and 0.35 gm, respectively, with these diets. Thus type of fiber as well as level of fiber in the ration markedly influenced M.F.N. excretion. This may be of particular importance with rapeseed meal where the high fiber content might induce a greater excretion of M.F.N. and consequently reduce the apparent protein and dry matter digestibilities of RSM. However, as described earlier, there is no evidence that differences in the fiber content of rapeseed meal (approximately 13.7-16.0%) have any marked influence on the digestibility of RSM. Drouliscos and Bowland (1969) suggested that lignin in RSM is arranged and distributed in such a way that protein is trapped by the fibrous material and not easily attacked by proteolytic enzymes. This suggestion

is in agreement with the observation of Whiting and Bezeau (1957a) that the level of fiber in the ration seems to interfere with the absorption of protein in addition to the increase in metabolic fecal nitrogen. Further experiments need to be carried out comparing the digestibility of low fiber (removal of the hull) and high fiber RSMs in order to answer the question of whether fiber interferes with absorption of rapeseed protein.

B. Feed intake and fecal output

Besides the effect of fiber, food intake and fecal output also can affect M.F.N. excretion. Blaxter and Wood (1951) concluded from their studies with calves that the output of dry feces rather than D.M. intake determined M.F.N. output. Meyer (1956) found that fecal dry matter appeared more satisfactory than D.M. intake as a reference base for the estimation of M.F.N. with the rat. Zelter and Charlet-Lery (1961) also reported a high correlation ($r = 0.90$) between M.F.N. and fecal D.M. excretion. This relationship was expressed by the equation

$$Y = 0.07X+0.85$$

where Y is the M.F.N. in gm and X is fecal D.M. output in gm. They observed that M.F.N. excretion by pigs decreased in a curvilinear fashion as feed intake increased and was therefore poorly related to feed intake. However there was a very high correlation ($r = 0.92$) between M.F.N. and the logarithm of dry matter ingested. The linear relationship between M.F.N. excretion (Y) and the D.M. intake (X^1) was expressed by the regression equation

$$Y = \log X^1 - 10.240$$

Thus the estimation of metabolic N losses from fecal D.M. excretion appears to be a better means of determining M.F.N. excretion. An increase in the dry matter excretion as a result of poor digestibility of a diet therefore would have an appreciable influence on M.F.N. losses. Zelter and Charlet-Lery (1961) concluded that a decrease of one percent in apparent dry matter digestibility increased M.F.N. by 4.1% per 100 grams of dry matter ingested and decreased it by 1.9% per 100 gm of dry matter excreted.

Metabolic fecal nitrogen output appears to vary directly with the logarithm of the feed intake or with the output of fecal D.M. Thus diets which result in low digestibility, such as that observed with rapeseed protein, are likely to induce a greater excretion of metabolic fecal nitrogen compared to diets which are readily digested. Thus, differences in excretion of M.F.N. should be taken into account when interpreting data on true and apparent digestibility of proteins.

C. Effect of protein level

The effect of level of protein on M.F.N. is also important in the interpretation of data on apparent and true protein digestibility. Values for M.F.N. are usually determined with a low protein or by extrapolation to zero from data obtained at different protein levels. Mitchell et al. (1954) postulated that M.F.N. was independent of the dietary protein level by showing that M.F.N. determined on a low-protein ration was the same as that obtained by extrapolation of series of nitrogen levels to zero protein levels. Similarly, Whiting and Bezeau (1957a) reported that differences in apparent digestibility of protein as a result of differences in levels of protein in the ration were due entirely to differences in

M.F.N. excretion. Although the apparent digestibility of the protein was markedly influenced by the level of protein in the ration, dietary protein levels had no effect on the true digestibility of proteins used in their study. Average apparent protein digestibility ranged upward from 78 to 87 percent for diets containing 5 to 22% protein whereas true digestibility for the same diet was constant at 89-91 percent.

However the validity of M.F.N. values has been questioned by several researchers (Titus, 1927; Bosshardt and Barnes, 1946; Wang and Grossman, 1951; Gadzieva, 1956; Sumi, 1958; Albanese, 1959; Booth et al., 1960; Twombly and Meyer, 1961; Abdeljil and Desnuelle, 1964; Snook, 1965) on the basis that level and source of protein can stimulate the enzymatic output of the pancreas (see chapter 2.3.2) and thereby influence the proportion of endogenous proteins secreted.

Recent findings by Carlson and Bayley (1970) seem to confirm the hypothesis that the metabolic nitrogen output varies with the level of protein in the diet and the digestibility of the protein. They found that the level of casein in the diet did not influence the corrected digestibilities of the proteins and amino acids whereas increasing the level of soybean meal in the diet resulted in a significant reduction in the true protein digestibility and true digestibility of amino acids such as isoleucine, leucine and proline. True digestibilities of soybean meal were 89, 86 and 85% at 7, 14 and 21% of protein, respectively, whereas true digestibility values for casein were constant at 98-99%. It seems therefore that the lower true digestibility of soybean meal at increasing level of protein is due to higher excretion of M.F.N. with a higher level of dietary protein.

Determination of M.F.N. using a low protein diet appears inaccurate because of the possibility that protein level and the source of protein influence the endogenous protein secretion. A protein-free diet probably underestimates the amount of M.F.N. as well as true digestibility coefficient. The precise determination of M.F.N. therefore requires extrapolation to zero of fecal nitrogen data obtained at different levels of protein with each particular protein.

2.4.4 Influence of microflora upon amino acid availability

Experiments on amino acid availability are open to criticism because microorganisms in the intestine may alter the amino acid distribution of food residues as they pass through the intestinal tract. Such an effect might be of particular importance in cases where relatively large amounts of proteins are poorly digested. The literature relating to microbial fermentation in the alimentary tract of the pig is somewhat contradictory; some researchers have found little effect of microbial action on amino acids patterns or breakdown in digesta although there are numerous reports dealing with fermentation of protein and amino acids in the lower part of the gut.

Microbial fermentation in the alimentary tract of the pig has been reviewed by Cranwell (1968). It appears that fermentation of carbohydrates, lipids and proteins takes place in all regions of the pig intestine, commencing with the first week of life. The role of the large intestine in the breakdown of nondigested protein residues has been extensively studied with normal, axenic and "germ free" rats (Gorden, 1959; Levenson and Tennant, 1963; Luckey, 1963; Combe et al., 1965; Combe and Pion, 1966; Harmon et al., 1968; Lepkovsky et al., 1966)

Levenson and Tennant (1963) reported that fecal excretion of nitrogen with axenic rats was twice that of normal rats. Similarly Luckey (1963), Combe et al. (1965) and Lepkowsky et al. (1966) observed that fecal nitrogen excretion was about 30% greater in axenic than in normal rats. Caecectomized animals also appear to excrete a greater amount of protein than normal animals. Nesheim and Carpenter (1967) found that the digestibility of heat damaged cod muscle was 77% in intact chicks compared to 68% for caecectomized birds. However there was no difference in the apparent digestibility (90 and 89% respectively) of non-heat damaged cod muscle in either intact or caecectomized birds. The authors attributed the lower digestibility of heat damaged cod muscle in caecectomized birds to the fact that these birds were unable to breakdown residual protein because of the absence of the caecal microflora. Recently, Salter and Coates (1971) studied the course of digestion of ^{14}C-labeled egg albumin that had been either freeze-dried or heat damaged and found consistently higher ^{14}C: nitrogen ratios in digesta from the large intestine of conventional birds than in germ-free birds. THey concluded that a greater breakdown and deamination of amino acids had occurred in the caecum and the colon of normal birds.

Influence of bacterial fermentation on amino acid availability in the rat was investigated by Kuiken (1952). He found only a slight reduction in digestibility of total nitrogen and amino acids when expeller cottonseed meal was fed in a ration which contained 2% succinyl sulfathiazole. Arginine, leucine, phenylalanine, tryptophan and valine availabilities were slightly lower (2-3%) in the presence of sulfathiazole whereas there was a slight increase (1%) in the availability of methionine.

By contrast several workers have suggested that microflora appreciably alter the pattern and digestibility of amino acids. Payne et al. (1968) found that caecectomy in the chick lowered the apparent digestibility of isoleucine by 10% and other amino acids by about 5%. Michel (1961, 1965, 1966, 1968) investigated the in vitro destruction of amino acids by microflora taken from the pig at slaughter and incubated under conditions closely resembling those in the intestinal lumen. Intestinal flora, in particular those of the ileum and caecum, were found capable of catabolizing all the amino acids but the rate of breakdown depended on the particular amino acid. The rates of breakdown (% amino acid destroyed in 42 hours of incubation) were 100 for serine and glutamic acid, 98 for histidine, 78 for aspartic acid and 62 for threonine. The other amino acids were broken down more slowly; alanine (50), leucine (52), isoleucine (42), valine (34), tyrosine (22), lysine (21), methionine (20), tryptophan (15). Only the L-form of the essential amino acids were broken down by the microflora except in the case of D-methionine. D-forms of the non-essential amino acids were broken down but more slowly than the L-forms. Except for the dicarboxylic amino acids, aspartic and glutamic acid, the deamination of the amino acids to give ammonia and organic acids was generally more rapid than decarboxylation to carbon dioxide and amines.

Decarboxylation of lysine, histidine, arginine, tyrosine and tryptophan was found in the ileum of pigs with the primary products of fermentation being amines such as cadaverine, histamine, putrescine, tyramine and tryptamine (Larson and Hill, 1960; Michel, 1966). Rate of breakdown also was found to depend on region in the intestine and

the type of diet. In general, catabolic activity of the flora was greater in the caecum and the colon than in the ileum and jejunum (Michel, 1965). However, the breakdown of some amino acids such as aspartic acid and cysteine occurred mainly in the ileum while arginine was degraded primarily in the caecum of pigs. The presence of carbohydrate in the gut affected the rate of amino acid catabolism. In the absence of carbohydrates bacteria will use amino acids for energy purposes. The catabolic activity in the caecum and colon also is proportional to the concentration of small peptides or free amino acids in these regions. This is confirmed by the observations that rapid intestinal transit such as that which accompanies diarrhoea (Michel, 1966) and low digestibility of a protein (Nesheim and Carpenter, 1967) appears to result in a greater amino acid breakdown in the lower region of the gut than that found with a normal intestinal transit or a very digestible protein.

Cho and Bayley (1972) observed no significant difference in the proportion of valine, arginine, serine, tyrosine, threonine, phenylalanine and aspartic acid in digesta from the ileum and rectum of swine fed soybean meal or rapeseed meal. The similarity in the proportion of these amino acids in the digesta from these two regions suggests that analysis of fecal samples probably reflects the digestibility of these amino acids. However, the proportion of isoleucine and methionine, in digesta from soybean meal, increased significantly between the ileum and rectum whereas the proportion of proline, leucine + glycine and glutamic acid decreased significantly. With rapeseed meal similar changes were found in the proportion of isoleucine, proline, leucine + glycine and

glutamic acid between the ileum and rectum and in addition there was a significant decrease in the proportion of alanine and a significant increase in that of lysine. These changes in the proportion of certain amino acids, between ileum and rectum, might lead to an overestimation or underestimation of amino acid availability from the proteins under investigation. The authors suggested that the high digestibility coefficients previously reported for proline and glutamic acid (Cho and Bayley, 1970; Giovanetti et al., 1970) was probably associated with the bacterial breakdown of these amino acids in the lower part of the alimentary tract. The low digestibility of methionine and alanine in soybean meal (Sauer , 1972) is probably associated with the lower catabolism of these amino acids in the caecum of pigs.

These observations by Michel (1961, 1962, 1966, 1968) and Cho and Bayley (1972) suggest that microflora can modify the amino acid pattern of digesta in the lower part of the alimentary tract. Fecal analysis may therefore overestimate or underestimate the availability of some amino acids. These findings support the suggestion of Payne et al. (1968) that amino acid content determined from ileal digesta samples probably give a more accurate index of amino acid availability than that based on fecal samples.

Chapter III

MATERIAL AND METHODS

3.1 Aim and design of the experiments

The primary object of the present study was to assess the nutritional quality of RSM produced in Canada and to investigate the reason(s) for the consistently lower digestibility of protein in RSM compared to that of other protein sources. The first experiment (Experiment I) investigated the effect of processing and variety of RSM upon various parameters of protein utilization such as digestibility of dry matter and nitrogen, amino acid availability, nitrogen retention, feed intake and weight gain. The plan of the experiment was a completely randomized block design where six piglets per group were assigned to twelve different RSM treatments. The RSM samples were supplied by 5 different processors (Table 1) under the Rapeseed Utilization Assistance Program and were crushed during July to October 1968 but were from seed produced during 1967 and 1968. These samples were from two main varieties, Brassica campestris and Brassica napus. They were commercially processed by solvent, prepress-solvent or expeller methods. Also included were samples from zero-erucic acid seed of B.napus (Sample 1) and from a low-thioglucoside content B.napus variety Bronowski (Sample 4).

Experiment II (Table 2) was carried out to compare digestibility, pattern of digestion and protein retention of RSM compared to other protein sources. The effect of amino acid supplementation of the various proteins on their digestion and utilization also was investigated. The second experiment was a 2 x 2 factorial involving 7 different protein sources each fed with and without amino acid supplement designed to compensate for the limiting amino acids in the various protein sources. A group of three piglets receiving a protein-free diet was also included to determine M.F.N.excretion and amino acid pattern of endogenous protein in digesta and feces.

Table 1. Description of rapeseed meal samples used in Experiment I.

Sample No.[1]	Manufacturing process[2]	Processor	Seed Type	Run[3]
1	Solvent	SWP	B.napus	A
2	Solvent	SWP	B.campestris	L
3	Solvent	SWP	B.campestris	E
4	Solvent	SWP	B.napus (Bronowski)	Br
5	Prepress-solvent	AGRA	B.campestris	N
6	Prepress-solvent	AGRA	B.campestris	B
7	Prepress-solvent	WCSP	B.campestris	F
8	Prepress-solvent	WCSP	B.campestris	P
9	Prepress-solvent	CVO	B.napus	S
10	Prepress-solvent	CVO	B.napus	D
11	Expeller	CLS	B.campestris	C
12	Expeller	CLS	B.campestris	H

[1]Samples 1 and 4 were produced from seed not available at that time for commercial use. The other samples were produced from commercial seed.

[2]SWP = Saskatchewan Wheat Pool, Saskatoon.
AGRA = Agra Vegetable Oil, Nipawin.
WCSP = Western Canadian Seed Processors, Lethbridge.
CVO = Co-op. Vegetable Oils, Altona.
CLS = Canada Linseed Oil Mills, Montreal.

[3]Identification of samples supplied under the Rapeseed Utilization Assistance Program (R.U.A.P.).

Table 2. Design of Experiment II

	Protein Source																			
	Casein		Auto-claved Casein		Soybean Isolate		Zein		RSM-1		RSM-4		RSM-5		Protein Free					
Amino acids	0[1]	+[2]	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	
No.of piglets	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3			
Total no. of piglets	4		4		4		4		4		4		4		3					

[1]no amino acid supplement

[2]with amino acid supplement - Type and level of amino acid supplements are given in Table 6.

A third experiment (Experiment III) was carried out to study the influence of fiber level on digestibility and utilization of soybean and RSM proteins. The experimental design (Table 3) was a 2 x 2 factorial with fiber level and protein source being the two main factors. High and low fiber RSM samples were obtained from three different processors. In each case the high and low fiber meals were prepared from the same seed source, viz. B. campestris, B. napus and Bronowski. In the low fiber RSMs the seed hull was removed by air classification (Bower Bros., N.Y.). Soybean meal was a commercially produced solvent meal containing 44% protein whereas low fiber soybean meal was a soybean isolate purchased from Nutritional Biochemicals Co., Cleveland. A total of 40 piglets, fiver per group, were used in the experiment.

In Experiment IV, in vitro pepsin, pepsin-pancreatin and pancreatin digests were carried out with some of the protein sources used in Experiments II and III, i.e. casein, autoclaved casein, isolated soybean protein, soybean meal, zein, and RSM-1. The purpose of the in vitro digestions was to study the relationship between rate of in vitro hydrolysis of the proteins and the rate of in vivo digestion of these proteins by piglets.

3.2 Composition of the diets

A semi-purified diet in which the various proteins served as the sole source of dietary nitrogen was used in all three experiments. The basal diet was composed of glucose, cellulose, soybean oil, rock phosphate, vitamins and mineral mix. Chromic oxide (Cr_2O_5) and polyethylene glycol (PEG 4000, J.T. Baker Co.) each added at 0.25% of the diet were used as internal markers in the last two experiments. Diets were mixed, 40 kg per lot, in a Hobart kitchen mixer and pelleted through a 0.32 cm die using a Templewood laboratory pelleting machine.

Table 3. Design of Experiment III.

Fiber Level[1]	Protein Source and Distribution of Pigs			
		Rapeseed (Seed Source and Processor)		
	Soybean	B. campestris WCSP[2]	B. napus CVO[3]	B. napus var. Bronowski SWP[4]
High	5	5	5	5
Low	5	5	5	5

[1] High fiber RSMs and SM were commercial meals.

Low fiber RSMs were prepared by air classification of the oil extracted residue while low fiber SM was a protein isolate purchased from Nutritional Biochemical Co., Cleveland.

[2] WCSP = Western Canadian Seed Processors, Lethbridge

[3] CVO = Co-op Vegetable Oils, Altona

[4] SWP = Saskatchewan Wheat Pool, Saskatoon.

The composition of the test diets used in the first experiment is given in Table 4. RSM made up 42.5% of the total diet. No attempt was made to adjust the nitrogen content of the various diets. The protein content of the diets ranged from 15.38 to 16.46% except for the diet containing Bronowski meal which contained 18.05% protein. In the protein-free diet RSM was replaced by glucose and cellulose, 27.5 and 15%, respectively.

Composition of the diets used in the second experiment is presented in Tables 5 and 6. Dietary protein sources included casein, autoclaved casein, soybean isolate, zein and three samples of RSM. The RSM samples were chosen on the basis of results obtained in Experiment I; RSM-1 was a low erucic acid variety of B. napus whereas RSM-5 was a variety of B. campestris. Apparent digestibility of protein tended to be higher for RSM-1 than RSM-5. RSM-4 was a low glucosinolate content meal (Bronowski) which had given superior performance in Experiment I. Similar diets were prepared by supplementing each of the protein sources with the most limiting amino acid(s) (Table 6). Amino acid addition was made on the basis of amino acid requirements of 6 weeks old piglets fed a diet containing 16% protein (Becker, 1958; Agric. Res. Council, London, 1967). Protein content (N x 6.25) of the diets varied from 15.50 to 16.02% with the non-supplemented diet (Table 5) and from 15.78 to 16.40 for the same diets supplemented with amino acids (Table 6). Less cellulose was added to the RSM diets than to other diets (10 vs. 15%) because of the high proportion of fiber in RSM. The protein-free diet was similar to the other diets except that it contained a high level of glucose and 20% cellulose.

Table 4. Composition of RSM and protein-free diets in the first piglet experiment

Ingredients	Diets and Levels of Ingredients (g/100 gm diet)	
	RSM diets	Protein-free diet
RSM	42.50	-
Glucose	45.50	73.00
Cellulose	5.00	20.00
Soybean oil	4.00	4.00
Vitamin mix[1]	1.00	1.00
Mineral mix[2]	0.50	0.50
Rock phosphate	1.50	1.50

[1]The vitamin mix supplied the following per 100 kg diet: Vitamin A, 250,000 I.U.; Vitamin D, 22,000 I.U.; dl-alpha tocopherol, 2,670 I.U.; menadione, 2.0; thiamin. HCl, 135 mg; riboflavin 0.33 gm; calcium pantothenate, 1.32 gm; pyridoxine HCl, 0.11 gm; choline chloride, 110 gm; Vitamin B_{12}, 3000 m.eq.1 gm; 0.75 gm; d-biotin, 8 mg; folic acid, 80 mg.

[2]The mineral mix supplied the following per 100 kg diet: sodium chloride, 465 gm; manganese oxide, 8.5 gm; zinc oxide, 1.0 gm; ferrous sulfate heptahydrate, 3.05 gm; copper sulfate pentahydrate, 2.5 gm; potassium iodine, 26 mg.

Table 5. Composition of diets used in Experiment II.

Ingredients	Diets and Levels of Ingredients (gm/100 gm diet)							
	Casein[1]	Autoclaved Casein[2]	Soybean Isolate[3]	Zein[4]	RSM-1[5]	RSM-4[6]	RSM-5[7]	Protein Free
Protein source	18.00	18.37	18.00	18.00	41.54	46.83	38.14	
Glucose	59.50	59.13	59.50	59.50	40.96	35.67	44.36	72.50
Cellulose	15.00	15.00	15.00	15.00	10.00	10.00	10.00	20.00
Soybean oil	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Vitamin mix[8]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mineral mix[9]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Rock phosphate	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Chromic oxide	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
PEG[10]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
N x 6.25	15.67	15.70	15.50	15.53	15.66	15.69	16.02	0.42

[1]Vitamin free casein, Nutritional Biochemicals Co., Cleveland, Ohio.
[2]Vitamin free casein autoclaved at 15 psi for 12 hrs with 2% glucose.
[3]Alpha protein, Nutritional Biochemicals Co., Cleveland, Ohio.
[4]Zein, Nutritional Biochemicals Co., Cleveland, Ohio.
[5]Rapeseed meal, B. napus (var. Oro), solvent processed, Saskatchewan Wheat Pool, Saskatoon.
[6]Rapeseed meal, B. napus (var. Bronowski), solvent processed, Saskatchewan Wheat Pool, Saskatoon.
[7]Rapeseed meal, B. campestris (prepress solvent processed, AGRA Vegetable Oil, Nipawin).
[8]Vitamin mix, same as described in Table 4.
[9]Mineral mix, same as described in Table 4.
[10]PEG 4000 - polyethylene glycol, 4000 M.W. J.T.Baker Chemical Co., analyzed reagent grade.

Table 6. Type and level of addition made to amino acid supplemented diets in Experiment II.

Amino Acid Supplement[1]	Protein Source						
	Casein	Autoclaved Casein	Soybean Isolate	Zein	RSM-1	RSM-4	RSM-5
DL-methionine[2]	0.20	0.30	0.30	0.30	-	-	-
L-lysine[3]	-	0.20	-	0.60	0.25	0.25	0.25
L-tryptophan[4]	-	-	-	0.06	-	-	-
N x 6.25	15.78	16.13	15.78	16.40	15.90	16.30	16.00

[1]Amino acids were added at the expense of glucose. Levels of the other ingredients were the same as described in Table 5.

[2,3,4]Amino acids from Nutritional Biochemical Co., Cleveland, Ohio.

Composition of the diets used in Experiment III is given in Table 7. Level of cellulose was adjusted in an attempt to euqalize the fecal dry matter excretion by the piglets fed the different diets. The proportion of glucose in low fiber RSM also was adjusted in order to maintain all diets more or less isonitrogenous.

3.3 Digestibility studies

Male castrated piglets, 5 to 8 weeks of age weighing approximately 11 kg were randomly assigned to various dietary treatments and housed in circular metabolic cages (Figure I) described by Bell (1948). Weight of the piglets was recorded at the beginning and the end of each collection period. In the first digestibility experiment the adjustment period was 3 days and collection period 4 days. In the two subsequent experiments the adjustment period was extended to 4 days and the collection period to 5 days. Lengthening the adjustment and collection periods was made in order to permit the pigs to adjust to the low palatability of RSM diets and to attempt to decrease the variation in digestibility coefficients observed in the first experiment.

In the first and third experiments piglets were fed the experimental diets ad libitum for a 1 hour period at 9 a.m. and 5 p.m. daily. Individual feed consumption at each meal was limited to a maximum equivalent to 2.5% of the liveweight of piglets in an attempt to avoid excessive difference in feed intake among treatments. Water was available at all times.

Feces were collected once daily. One percent ferric oxide was added to standard rapeseed meal (RSM) or soybean meal (SM) diets which were fed prior to and following the collection period, thereby facilitating the identification of the beginning and the end of the

Table 7. Composition of diets used in Experiment III.

	Diets and Levels of Ingredients (gm/100 gm diet)							
			Rapeseed					
	Soybean		B.campestris (WCSP)		B.napus (CVO)		B.napus (var.Bronowski,SWP)	
Ingredients	Meal	Isolate[1]	Meal	Low Fiber	Meal	Low Fiber	Meal	Low Fiber
Protein source	35.00	18.00	42.50	40.97	42.50	39.04	42.50	34.27
Glucose	47.00	59.50	45.00	41.53	45.00	43.46	45.00	48.23
Cellulose	10.00	15.00	5.00	10.00	5.00	10.00	5.00	10.00
Soybean oil	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Vitamin mix[2]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mineral mix[2]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Rock phosphate	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Chromic oxide	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
PEG	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Chemical Analyses (N x 6.25)	16.62	15.50	16.37	16.58	15.40	16.30	17.64	16.85
Crude Fiber[3] (% of the oil-free meal)	5.32	-	13.32	8.86	13.02	6.30	13.27	5.95

[1]Alpha protein, Nutritional Biochemicals Co., Cleveland, Ohio.

[2]Vitamin and mineral mix same as described in Table 4.

[3]Crude fiber according to A.O.A.C. (1965).

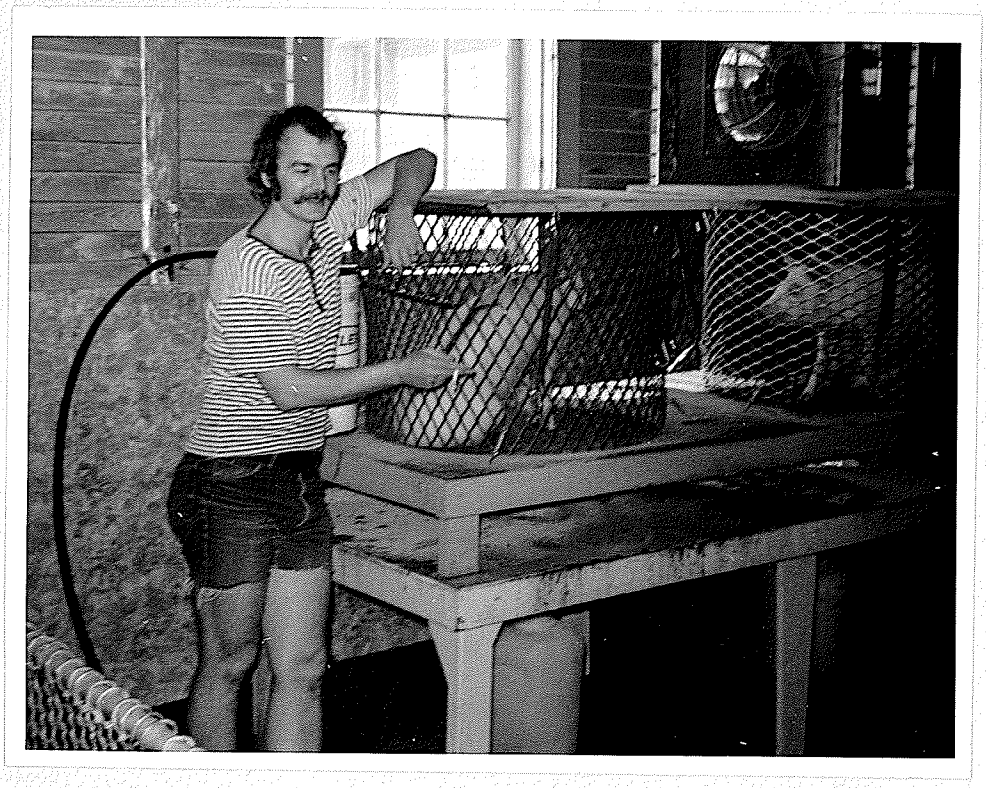

Figure 1. Circular metabolic cages used in digestibility trials.

collection period. Feces were stored in a refrigerator ($0^{o}C$) throughout the test period. The combined fecal collection was dried in a forced air oven at $70^{o}C$ for 48 hours. The dried samples were allowed to equilibrate with air, weighed and ground through a 40 mesh screen in a Wiley mill, model 3. An aliquot was then taken, placed in a plastic bag and stored in a dry place until analyzed.

Urine collection was started 4 hours after the ingestion of the first test meal and continued until 12 hours after the ingestion of the last meal. Urine was collected in large plastic bottles containing 25 ml of 0.1 N H_2SO_4 and 10 ml toluene. Total daily urine excretion was measured and a 5% aliquot stored at $0^{o}C$. The daily aliquots were combined, filtered through glass wool and stored at $-20^{o}C$ for further analyses.

True digestibility coefficients were calculated from metabolic fecal nitrogen (M.F.N.) excretion by piglets fed a protein-free diet containing 20% of cellulose. In the first experiment 8 piglets were used in the determination of M.F.N. whereas in the second experiment only three piglets were used. All procedures with piglets fed the protein-free diet were the same as described for the test group. Metabolic fecal nitrogen (M.F.N.) excretion in the first experiment was based on feed intake whereas in experiments 2 and 3, M.F.N. was estimated on the basis of fecal output. Details on calculations of true digestibility of protein will be given in the section "Calculations".

3.4 Blood collection

Blood plasma was obtained for the determination of free amino acids during Experiment II. Three samples of blood were taken from

the jugular vein of each piglet on the fourth day of experiment. The first sample was withdrawn just prior to feeding following a 14 hours fast, and the other samples were taken 1 hour and 3 hours after completion of the 1 hour feeding period. Heparin was used as an anticoagulant. The blood was centrifuged immediately after collection at 2000 x g for 20 minutes and the plasma transferred to a screw-cap vial and stored at -20°C until analyzed for free amino acids.

3.5 Collection of digesta samples

Digesta samples for the stomach, duodenum, upper jejunum, mid jejunum, lower jejunum, ileum, caecum, and rectum were collected from each piglet during the 2nd and 3rd experiments. The pigs were killed by electrocution approximately 4 hours after a meal of the test diets on the day following the completion of the collection period. Samples of digesta were quickly collected by gently extruding into plastic bags and immediately frozen in liquid nitrogen. The samples were lyophilized within 72 to 96 hours of collection, finely ground in a mortar and stored at -20°C until analyzed.

3.6 *In vitro* digests

The method used for the *in vitro* digests was similar to that described by Akeson and Stahmann (1964). Pepsin digests were prepared by incubating with continuous shaking, approximately 1 gram of protein from each source with 40 mg of pepsin (hog stomach, U.S.P. 1:10,000; Mann Research Laboratories) in 50 ml of 0.1 N HCl.

Incubations were carried out for 0, ½, 1, 2, 4, 16, and 32 hours at 37°C. Pepsin-pancreatin digests were prepared by incubating 1 gm of protein from each source with 15 mg pepsin in 25 ml of 0.1 N HCl for 4 hours at 37°C. The samples were then neutralized with NaOH, made 0.2 M with phosphate buffer (pH 8.0) and incubated with 40 mg pancreatin (hog, 3 x N.F., Mann Research Laboratories) for a further 0, 1, 2, 4, 8, 16, and 32 hours. Pancreatin digests were prepared by incubating 1 gm protein with 40 mg pancreatin in 50 ml of 0.2 M phosphate buffer (pH 8.0) for 0, ½, 1, 2, 4, 8, 16, and 32 hours. Blanks at the beginning and the end of each set of digestions were obtained by incubating the protein sources under the conditions described but with the enzyme omitted. All digests were covered with 2 ml of toluene in an attempt to prevent microbial fermentation. Following incubation the samples were immediately cooled in an ice bath and centrifuged at 14,000 x g for 15 minutes at 0°C. Nitrogen was determined in the supernatant before and after precipitation of the soluble protein with sulfosalicylic acid (5% final concentration W/V). Amino nitrogen was estimated by formol titration of the soluble supernatant. Patterns of total and free amino acids were determined on the deproteinized supernatant from a 5 hours pepsin-pancreatin digest (4 hrs pepsin and 1 hr pancreatin).

3.7 Chemical Analyses

3.7.1 Nitrogen

Total nitrogen in the diets and feces was determined by the

macro-Kjeldahl method (A.O.A.C., 1965) whereas total nitrogen in freeze-dried digesta samples was determined by the micro-Kjeldahl method (A.O.A.C., 1965). Solubility of nitrogen in digesta samples was determined by the following method: freeze-dried digesta sample (1 gm) was mixed with cold water (100 ml at $4^{o}C$) with a Polytron blender for 5 minutes; the homogenate was then centrifuged 15 minutes at 15,000 x g at $0^{o}C$ according to the method of Snook and Meyer (1964a); and the supernatant (soluble nitrogen) and protein residue (insoluble nitrogen) were analyzed for nitrogen by the micro-Kjeldahl method (A.O.A.C., 1965).

3.7.2 Chromic oxide and PEG determination

Dried samples from diet, digesta and feces were analyzed for chromic oxide (Cr_2O_3) by the method of Brisson (1956) using perchloric acid as an oxidizing agent and sodium molybdate as a catalyst. Optical densities of solutions were determined at a wave length of 444 mμ using a Bausch and Lamb, model Spectronic 20, spectrophotometer. Standard curves were obtained by treating pure Cr_2O_3 (0, 10, 20, 30 and 50 mg per reaction vessel) in the same manner as that used for diet and digesta samples. A plot of optical densities against concentration of Cr_2O_3 was a straight line in the range of 0 to 40 mg Cr_2O_3.

Polyethylene glycol (PEG 4000 M.W.) was determined by a modification of the turbidimetric method of Hyden (1956). In the present study PEG was extracted from dry samples with hot water (95-$100^{o}C$) whereas Hyden (1956) extracted PEG directly from the clear supernatant by filtration or centrifugation of moist digesta samples. Dried samples were weighed (250-500 mg) directly in 25 ml glass centrifuge tube fitted with a screw cap. Ten ml of water was added and the tubes were placed

in a hot water bath for 20 minutes. The contents of the tubes were mixed at 5 min. intervals with a Vortex hand mixer. The tubes were allowed to cool and centrifuged at 2500 x g for 20 minutes. Concentration of the PEG in the resulting clear supernatant was determined by the procedure described by Ulyatt (1964). PEG was found unsatisfactory for digestibility determinations with RSM. A similar observation was reported by Cho and Bayley (1972). The presence of tannins in RSM may account for the low recovery of PEG in digesta and feces of animals fed RSM. Tannins in heather have been found to precipitate PEG along with protein in the rumen of deer (Kay, 1969b).

3.7.3 Amino Nitrogen

Amino nitrogen was estimated in samples of digesta obtained from the digestive tract and in *in vitro* digests by formol titration using the procedure described by Van Slyke and Kirk (1933). Ten ml of soluble supernatant was adjusted to pH 9.0 using 0.2 N NaOH. Ten ml of 40% neutral formaldehyde (Baker Analyzed Reagent, Phillipsburg, N.J.) was added and the solution titrated with 0.1 N NaOH to pH 9.0. Ten ml of a 0.1 M glycine solution was used as a standard. Amino nitrogen was expressed either as a percent of soluble nitrogen or total nitrogen (N x 6.25).

3.7.4 Amino acid analyses

a. Diet, digesta and feces

Hydrolysis of diets, digesta and feces for amino acid analyses was carried out in approximately 500 ml Mason jars containing 1 gm of sample and 100 ml of 6 N HCl. The jars were flushed with nitrogen,

sealed and heated in an oven at 105°C for 22 hours. No special oxidative hydrolysis method was used for methionine and cystine determinations. An aliquot of hydrolyzate was evaporated to dryness on a rotary flash evaporator. The amino acids were redissolved in 0.2 M Na citrate buffer, pH 2.2. This solution was filtered through a sinter glass funnel (medium porosity) and the amino acids determined by ion exchange chromatography using a Beckman Model 117 amino acid analyzer equipped with a Model 138 automatic sample injector and a Beckman Model 125 integrator according to the method of Benson and Patterson (1965a). Amino acid concentrations in the samples were computed by comparing them with analyses of standard mixtures of amino acids. A lower recovery of ninhydrin-positive compounds (including NH_3) was found in samples of digesta and feces as compared to the diets. Thus the results of the 2nd and 3rd experiments were standardized to 95% recovery as recommended by Knipfel et al., 1971. This procedure reduced considerably the variations in amino acid concentrations (gm/16 gm N) associated with differences in recovery of amino acids.

b. Plasma amino acids

Frozen plasma was thawed and a 4.0 ml aliquot was mixed with 4.0 ml of 0.2 M Na citrate buffer, pH 2.2, and deproteinized using 8.0 ml of 9% W/V sulfosalicylic acid. The solution was centrifuged at 30,000 x g for 10 minutes and the clear supernatant removed. The protein residue was washed with an additional 4.0 ml of buffer and recentrifuged. The combined supernatants were then evaporated to dryness on a rotary flash evaporator and the residue redissolved in 4.0 ml glass distilled water. Plasma amino acid analyses were carried out on a Beckman Model 117 amino acid analyzer using the procedure for physiological fluids described by Benson and Patterson (1965b).

c. Free amino acid in pepsin-pancreatin digests

Free amino acids were determined in the clear supernatant of pepsin-pancreatin digests (4 hrs pepsin, 1 hr pancreatin), following precipitation of the soluble protein with 5% sulfosalicylic acid (W/V). The clear supernatant obtained by centrifuging at 30,000 x g for 10 minutes was diluted with an equal volume of 0.2 M Na citrate buffer, pH 2.2, and the free amino acids determined by the ion exchange method described for protein hydrolyzates (Benson and Patterson, 1965a). Amino acid composition of the same supernatant fraction also was determined following hydrolysis with HCl. Release of the individual amino acids was expressed either as percent of each amino acid in the 5% sulfosalicylic supernatants or as percent of each amino acid in the protein.

3.7.5 Trypsin and chymotrypsin assays

Trypsin and chymotrypsin activities were determined on the soluble nitrogen fraction in digesta from the mid jejunum. The soluble nitrogen fraction was obtained according to the procedure described previously for determination of soluble nitrogen. Assay methods for trypsin and chymotrypsin were those of Hummel (1959). A Unicam SP-800 spectrophotometer equipped with a Model SP-20 recorder was used for all determinations. Assays were made at 30°C and reagents were kept at the same temperature in an independent water bath. Trypsin activity was determined by the rate of hydrolysis of p-toluene-sulfonyl-L-arginine methyl ester (TAME, Sigma Chemical Co., St. Louis, Missouri) measured by the change in absorbancy at 247 mμ (ΔA247). The test cuvette contained 3.00 μmoles of TAME, 0.12 mmole Tris buffer (pH 8.1 containing 0.030 mmoles of $CaCl_2$) and 0.1 ml of clear digesta supernatant in a final volume of 3.0 ml. The supernatant was adjusted to give a change in absorbence of less than 0.32 per minute. A blank

cuvette was prepared in the same manner as the test cuvette but contained 0.1 ml of a 0.001 N HCl solution instead of the supernatant. Trypsin activity per 100 mg of dry matter (D.M.) was calculated according to the following formula:

$$\text{Activity in units per 100 mg of D.M.} = \frac{\Delta \text{ A247} \times 3 \times \text{Dil.Factor}}{540}$$

where activity in units is the micromoles TAME hydrolyzed/min at 30°C, pH 8.1; Δ A247 represents the change in absorbance per minute in a test cuvette containing a final volume of 3.0 ml and the molar extinction coefficient for p-toluenesulfonyl L-arginine at 247 mμ is 540. The dilution factor usually was x 10^5.

Chymotrypsin activity was determined from the rate of hydrolysis of benzoyl-L-tyrosine ethyl ester (BTEE, Sigma Chemical Co., St.Louis, Missouri) as measured by the change in absorbance at 256 mμ (A256). The test cuvette contained 1.5μmole BTEE (in 50% W/V methanol), 0.12 mmole Tris buffer (pH 7.8, containing 0.15 mmole $CaCl_2$) and 0.1 ml of clear digesta supernatant in a final volume of 3.0 ml. The blank cuvette was the same as the test cuvette but contained 0.1 ml of 0.001 N HCl solution instead of the supernatant solution. Chymotrypsin activity was calculated according to the following formula:

$$\text{Activity in units per 100 mg D.M.} = \frac{\Delta \text{ A256} \times 3 \times \text{Dil.Factor}}{964}$$

where activity in units is the micromoles BTEE hydrolyzed/min at 30°C, pH 7.8; Δ A256 represents the change in absorb nce per minute in a test cuvette containing a final volume of 3.0 ml and the molar extinction coefficient for N-benzoyl L-tyrosine at 256 mμ is 964.

3.8 Calculations

3.8.1 Digestibility

Coefficients of apparent digestibility for protein and amino acids by the total collection method were calculated by the following formula:

$$\% \text{ apparent digestibility} = \frac{(I - E)}{I} \times 100$$

where I is the protein (N x 6.25) or individual amino acid intake and E is the fecal protein (N x 6.25) or individual amino acid excretion. When Cr_2O_3 was used as an index the formula was:

$$\% \text{ apparent digestibility} = 100 - \frac{F}{D} \times 100$$

where F is the ratio of protein or amino acid to Cr_2O_3 in the feces and D is the ratio of protein or amino acids to Cr_2O_3 in the diet. The apparent rate of absorption of protein and amino acids in the various segments of the intestine also was determined according to a similar formula.

True protein or amino acid digestibility by the total collection method was calculated by correcting for metabolic fecal nitrogen or metabolic amino acids. Metabolic fecal nitrogen (M.F.N.) or metabolic amino acid output was determined with 8 piglets given a protein-free diet for a period of 4 days. The mean M.F.N. was 0.177 gm or 1.104 gm of protein (N x 6.25) per 100 gm of feed intake. The formula used to calculate true digestibility of protein or amino acid by the total collection method was:

$$\text{True protein (or a.a.) digestibility} = \frac{(I - (E-M)) \times 100}{I}$$

where I is the protein (N x 6.25) or individual amino acid intake; E is the

protein (N x 6.25) or amino acid excretion and M is M.F.N. or the metabolic amino acid output based on feed intake. True protein or true amino acid digestibility coefficients as well as ratio of absorption also were determined using Cr_2O_3 as index material by the formula:

$$\% \text{ true protein (or a.a.) digestibility} = 100 - \frac{(F - PF)}{D} \times 100$$

where the F and D values are the ratios of protein (or a.a.) to Cr_2O_3 in the digesta or the feces and in the diet, respectively, while PF is the average ratio of protein (or a.a.) to Cr_2O_3 in the digesta or feces of 3 piglets (Experiment II) fed a protein-free diet.

3.8.2 Statistical analyses

Analysis of variance and co-variance and calculations of correlation coefficients were carried out according to the methods of Snedecor and Cochran (1967). Differences among treatments were compared by Duncan's multiple range test (Duncan, 1955) or using a test of significance between means of independent samples (Snedecor and Cochran, 1967).

Chapter IV

RESULTS AND DISCUSSION

4.1 Influence of source of rapeseed meal on protein utilization - Experiment I.

4.1.1 Dry matter and protein digestibility

No significant differences ($P<0.05$) were found in the digestibility of dry matter and protein (apparent or true) among 12 different RSMs from 5 different processors (Table 8). Dry matter digestibility for the various samples varied from 72.4±5.8 to 78.9±6.4 whereas the apparent digestibility of protein varied from 71.0±2.0 to 77.9±3.2. The digestibility of dry matter was highly correlated ($r = 0.90$) with the digestibility of the protein. The relatively high standard deviation values within the various diets groups reflect the marked variation observed among animals fed these sources of RSM. Neither processing nor variety of RSM had any appreciable effect on digestibility of D.M. or protein. The average digestibility for dry matter and protein of expeller processed RSMs was only slightly lower than that of prepress and solvent processed RSM. The apparent protein digestibility coefficients for expeller RSMs averaged 73.4 compared to 74.8 and 76.9% with prepress-solvent and solvent RSM, respectively. The digestibility of D.M. and protein for the low glucosinolate content RSMs (B. campestris and Bronowski meal) was similar to that of the high glucosinolate RSMs (B. napus).

These observations agree with those of Drouliscos and Bowland (1968) and Saben et al. (1971b) in that type of processing, variety of RSM and glucosinolate content (Table 8) of RSM has no influence on the digestibility of dry matter (D.M.) or protein.

Table 8. Digestibility coefficient of protein and dry matter in rapeseed meal samples.

Processing Method	Sample No.	Variety	Digestibility (%)		
			Dry matter	Protein	
				Apparent	True
Solvent	1[1]	B. napus	78.5±3.2[3]	77.8±4.0	84.6±4.0
	2	B. campestris	75.1±2.6	74.8±6.1	81.6±5.6
	3	B. campestris	78.9±6.4	77.9±6.8	85.0±6.9
	4[2]	B. napus (Bronowski)	75.8±1.7	77.1±4.1	82.3±2.4
Prepress-Solvent	5	B. campestris	72.4±2.4	71.0±2.0	78.2±2.1
	6	B. campestris	76.6±4.2	74.9±5.1	81.8±5.1
	7	B. campestris	74.6±3.0	74.9±3.1	81.7±3.1
	8	B. campestris	76.7±2.5	77.9±3.2	84.6±2.9
	9	B. napus	74.4±8.6	75.3±8.7	82.7±8.7
	10	B. napus	75.5±2.7	75.0±3.3	82.0±3.4
Expeller	11	B. campestris	74.8±8.1	73.4±1.9	80.1±1.9
	12	B. campestris	72.4±5.8	73.3±6.3	80.7±5.7

[1]Zero-erucic acid rapeseed meal.

[2]Low-thioglucoside content rapeseed meal.

[3]Mean±S.D. for 6 pigs.

4.1.2 Feed intake, weight gain and nitrogen retention

Unlike protein digestibility RSM varieties had a significant effect on feed intake (Table 9). In general, except for the Bronowski meal (Sample 4), piglets fed the Brassica napus varieties (Samples 1, 4, 9 and 10) had a lower feed intake than those fed the campestris varieties Feed intakes with samples 1, 9 and 10 were significantly ($P < 0.05$) lower than those with samples 4, 7 and 8. Weight gain and nitrogen retention followed a similar pattern to that of feed intake. The coefficients of correlation between weight gain and feed intake, and nitrogen retention and feed intake, were 0.62 ($P < 0.01$) and 0.76 ($P < 0.01$), respectively. Nitrogen retention was significantly ($P < 0.05$) lower with samples 9 and 10 than samples 4, 7 and 8. Analysis of covariance indicated that the feed intake was the main factor affecting nitrogen retention by piglets fed various sources of RSM. There were no significant differences among treatments in the efficiency of nitrogen retention expressed either in terms of percent of N intake or percent of apparent nitrogen absorbed. However, there was an appreciable relationship between total nitrogen retained and the efficiency of nitrogen retention. A correlation coefficient of 0.78 was found between total amount of nitrogen retained and nitrogen retained as percent of N intake. Similarly the correlation coefficient between total amount of nitrogen retained and nitrogen retained as percent of N absorbed was 0.78. Since there was a positive relationship ($r = 0.33$, $P < 0.01$) between feed intake and efficiency of nitrogen utilization (as percent of nitrogen absorbed) it would appear that the significant difference in nitrogen retention among the piglets fed the different RSM samples was mainly due to feed intake rather than efficiency of protein utilization.

Table 9. Feed intake, weight gain and nitrogen retention data for pigs fed rapeseed meal as the sole source of protein.

Processing Method	Sample No.	Variety	Feed Intake (gm/pig/4 days)	Weight Gain (Kg/pig/4 days)	Nitrogen retention		
					gm/pig/4 days	% N intake	% N absorbed
Solvent	1	B. napus[1]	1762±530[3] cde[4]	0.44±0.21 cde	19.9± 9.3 cd	41.8± 8.2	53.9±12.8
	2	B. campestris	2050±326 abcde	1.03±0.68 bc	24.3± 8.5 abcd	44.3±11.9	59.2±16.0
	3	B. campestris	2141±352 abc	1.14±0.61 ab	27.1± 8.4 abcd	49.6±13.5	63.0±12.1
	4	B. napus (Br)[2]	2505±344 a	1.74±0.59 a	32.1±10.3 ab	44.9±14.2	59.0±18.8
Prepress-Solvent	5	B. campestris	2141±491 abc	1.22±0.28 ab	21.2± 8.6 bcd	39.1± 9.5	55.2±14.1
	6	B. campestris	1948±406 abcde	0.80±0.47 bcd	20.3± 4.8 bcd	41.1± 8.8	54.6± 9.5
	7	B. campestris	2304±323 ab	1.35±0.50 ab	28.7± 4.1 abc	48.2± 6.1	64.6± 6.8
	8	B. campestris	2340±439 ab	1.16±0.62 ab	35.0± 8.8 a	54.7± 6.1	70.3± 6.0
	9	B. napus	1565±333 e	0.03±0.46 e	15.4±11.2 d	38.3±21.4	49.2±22.7
	10	B. napus	1522±454 e	0.26±0.11 de	17.1± 6.0 d	45.1± 4.5	59.3± 5.9
Expeller	11	B. campestris	1818±389 bcde	0.36±0.46 cde	26.9± 3.5 bcd	46.7± 7.9	58.9± 2.5
	12	B. campestris	2168±584 abc	0.92±0.48 bcd	24.0±10.1 abcd	42.2±13.0	57.0±14.9

[1]Zero-erucic acid rapeseed meal, SWP.

[2]Low-thioglucoside content rapeseed meal.

[3]Mean ± S.D. for 6 pigs.

[4]Any two means not followed by the same letters are significantly different ($P < 0.05$).

It appears therefore that the lower performance (weight gain and nitrogen retention) of piglets fed high thioglucoside RSM (B. napus) was related to the lower feed intake and not protein digestibility or nitrogen utilization. It is interesting to note that Bell (1957), Belzile et al. (1963), Oliver et al. (1970) and Bell et al. (1971) also concluded that the poor performance of animals fed RSM was definitely associated with the thioglucoside level of the meal and its adverse effect on feed intake.

4.1.3 Availability of amino acids from rapeseed meals

Amino acid composition of the 12 RSM samples (Table 10) was similar. Neither source of meal (expeller, prepress-solvent or solvent RSM) nor the variety of the meal (Brassica napus vs. Brassica campestris) had any marked effect on the amino acid content, including the content of lysine and methionine, in the various meals. The lysine content of the meals ranged from 4.32 to 5.87; the highest value being for a prepress-solvent B. napus RSM (Sample 9) and the lowest for an expeller B. campestris RSM (Sample 12). The average lysine content (%) for the solvent, prepress-solvent and expeller RSM was 5.41±0.37, 5.19±0.52 and 4.69±0.51, respectively, while the lysine content of the B. napus RSM tended to be slightly higher, 5.44±0.40%, than that of the B. campestris meals, 5.05±0.51%. The proportion of methionine in the various samples ranged from 1.36% for a prepress-solvent RSM to 2.09 for a solvent processed RSM. The average methionine content of the expeller RSM was 1.91±0.14 compared to 1.76±0.22 and 1.77±0.14 for prepress-solvent and solvent RSM, respectively. The lack of a marked difference in the amino acid content among samples of RSM, particularly in the lysine and the methionine content, is in agreement with the findings of Clandinin

Table 10. Amino acid composition (gm/16 gm N) and protein level of the rapeseed meal diets used in Experiment I

	Method of Processing and RSM Samples												
	Solvent				Prepress-solvent						Expeller		
Amino Acids	1*[1]	2	3	4*	5	6	7	8	9*	10*	11	12	Mean± S.D.$_x$
Lysine	5.66	5.16	4.89	5.05	5.78	4.55	5.07	5.62	5.87	5.19	5.06	4.32	5.19±0.48
Histidine	2.84	2.42	2.62	2.92	2.71	2.32	2.65	2.87	2.52	2.32	2.58	2.70	2.62±0.20
Arginine	6.38	5.67	5.69	6.29	6.00	6.07	5.35	5.88	5.75	5.62	5.71	4.81	5.77±0.42
Aspartic acid	7.40	7.19	7.59	7.95	7.18	8.57	6.75	7.16	7.24	7.41	7.96	7.02	7.45±0.50
Threonine	4.15	3.83	4.56	4.11	4.29	4.40	4.14	4.27	4.18	3.95	4.02	4.30	4.18±0.20
Serine	4.57	4.18	4.64	4.58	4.32	4.46	4.19	4.21	4.43	4.02	4.25	4.60	4.37±0.20
Glutamic acid	18.22	15.97	18.61	17.52	17.10	15.58	16.43	17.55	16.46	17.34	15.74	18.99	17.13±1.12
Proline	6.42	6.01	6.01	4.88	6.15	5.61	6.29	6.86	6.08	5.65	6.05	5.38	5.95±0.52
Glycine	5.32	4.69	5.22	4.98	5.01	5.20	5.09	5.27	4.77	4.92	4.66	4.96	5.01±0.22
Alanine	4.92	4.53	4.48	4.65	4.81	4.37	4.36	4.60	4.85	3.95	4.45	4.32	4.52±0.27
Cystine	2.34	1.61	1.66	-	1.11	-	2.05	1.90	1.22	-	2.59	1.85	1.36±0.92
Valine	4.70	4.55	4.77	4.55	5.06	5.49	4.26	4.76	4.94	4.71	4.63	4.83	4.77±0.30
Methionine	1.76	1.79	2.09	1.64	1.92	1.76	1.36	1.89	1.67	1.81	2.01	1.81	1.79±0.19
Isoleucine	3.64	3.39	3.91	3.81	3.36	3.87	3.58	3.73	3.73	3.43	3.48	3.59	3.63±0.19
Leucine	6.94	6.73	7.65	7.16	6.88	7.11	7.02	7.33	5.46	6.43	6.66	7.01	6.87±0.54
Tyrosine	3.16	2.75	2.34	3.07	2.79	3.21	2.89	3.06	2.67	2.95	2.69	2.38	2.83±0.28
Phenylalanine	3.61	3.54	3.88	3.89	3.56	3.79	3.95	4.09	3.77	3.40	3.49	3.82	3.73±0.21
% Recovery of N[2]	94.0	84.5	91.6	87.7	86.2	85.9	85.8	92.9	87.8	83.8	86.3	82.6	87.4 ±3.67
% Protein in diets	16.23	15.45	15.87	18.05	15.43	16.05	16.46	16.14	15.35	15.38	15.58	16.05	16.0

[1]Samples marked with asterisk were prepared from B. napus seeds.

[2]$\% \text{ Recovery} = \frac{\Sigma \text{ N in Ninhydrin - Positive Compounds (including } NH_3) \times 100}{\text{Total N Applied to Column}}$

for the same samples (Giovanetti and Bell, 1971). Therefore we can conclude that some factor(s) other than processing or variety account for the differences in amino acid composition among these RSM samples.

Similarly processing and variety did not influence the amino acid composition of the feces (Table 11) of piglets fed the various RSM diets. In general, the levels of amino acids in feces when expressed as % of total N were lower than in the diet. This difference was due primarily to the lower apparent recovery of ninhydrin-positive compounds during the analysis of feces (71.7%) compared to the diet (87.4%). In addition the amino acid pattern in the feces differed significantly ($P < 0.05$) from that of the diet, particularly for arginine, histidine and glutamic acid where the content (gm/16 gm N) of these amino acids was only half that found in the diets. Similar observations were reported by Cho and Bayley (1970) for pigs fed RSM.

There were several similarities in the amino acid composition of the feces from piglets fed RSM when compared to those fed the protein-free diet, although the protein content of the feces from piglets fed the protein-free diet was considerably lower (8.68 vs. 16.32%) than that from animals fed the RSM diets. No significant difference was found in the proportion of histidine, arginine, serine, glutamic acid, glycine, cystine, valine, methionine, leucine and phenylalanine. Similarity in the content of some amino acids in the feces of animals fed the RSM and protein-free diets indicates either considerable contribution of metabolic protein to fecal amino acids or marked bacterial action on amino acids in the large intestine.

There were marked differences among the individual amino acids in the apparent and true digestibility (Tables 12 and 13). True digest-

Table 11. Amino acid composition (gm/16 gm N) of fecal samples collected from piglets fed rapeseed meal and protein-free diets.

	Method of Processing and RSM Samples													
	Solvent				Prepress-solvent						Expeller		RSM[2]	
Amino Acids	1	2	3	4	5	6	7	8	9	10	11	12	Mean±S.D.$_x$	Protein-free[3]
Lysine	5.30[1]	4.68	4.57	4.59	4.72	4.88	4.62	4.63	4.32	4.69	4.85	4.99	4.73±0.54	5.80±0.73
Histidine	1.47	1.50	1.37	1.48	1.58	1.35	1.45	1.53	1.34	1.70	1.36	1.53	1.46±0.24	1.61±0.48
Arginine	3.34	3.29	2.87	3.32	3.52	3.27	3.51	3.56	3.54	3.52	3.11	3.12	3.32±0.45	3.68±0.60
Aspartic Acid	7.74	6.94	7.38	7.12	7.54	7.62	7.43	7.27	6.52	6.79	6.68	6.83	7.15±0.76	8.66±1.01
Threonine	4.35	3.79	3.96	3.54	4.11	4.10	3.75	4.01	3.64	3.80	4.18	3.79	3.92±0.46	4.52±0.78
Serine	3.98	3.48	3.70	3.22	3.60	3.90	3.52	4.01	3.74	3.54	3.54	3.45	3.61±0.46	3.98±0.51
Glutamic acid	9.18	8.48	8.68	8.29	8.77	8.14	7.86	9.01	8.05	8.21	7.30	8.29	8.35±1.20	9.25±0.87
Proline	5.56	5.03	4.63	4.75	5.33	4.93	7.27	5.64	4.89	4.67	5.47	5.44	5.29±1.02	3.96±0.43
Glycine	4.73	4.22	4.32	4.05	4.48	4.82	4.62	4.49	4.21	4.19	4.47	4.44	4.41±0.45	4.61±0.47
Alanine	5.07	4.46	4.72	3.94	4.54	4.90	4.73	4.65	4.29	3.69	4.75	4.46	4.51±0.59	5.52±0,37
Cystine	0.50	0.64	1.00	0.65	0.84	0.70	0.92	0.51	0.39	0.86	0.54	1.51	0.75±0.55	0.77±0.78
Valine	4.82	4.13	4.31	4.09	4.43	4.43	4.24	4.47	3.92	4.07	4.61	4.20	4.30±0.50	4.94±0.84
Methionine	1.65	1.33	1.34	1.48	1.28	1.40	1.71	1.48	1.22	1.44	1.19	1.53	1.41±0.28	1.51±0.38
Isoleucine	3.67	3.18	3.38	3.32	3.46	3.45	3.44	3.31	3.00	3.17	3.24	3.66	3.35±0.41	3.74±0.48
Leucine	6.21	5.72	5.54	5.56	5.86	5.80	5.88	5.54	5.10	5.41	5.40	5.39	5.60±0.61	6.22±0.83
Tyrosine	2.92	2.62	2.66	2.76	2.78	2.77	3.05	2.69	2.41	2.84	2.66	2.59	2.72±0.40	3.27±0.40
Phenylalanine	3.71	3.14	3.18	3.23	3.29	3.24	3.30	3.22	2.90	3.18	3.08	3.31	3.22±0.43	3.60±0.44
% Recovery of N[4]	74.6	70.9	72.2	69.8	72.7	73.8	74.8	72.1	68.7	70.7	68.7	71.7	71.7 ±4.8	77.8 ±4.7
% Protein in feces	17.07	16.62	16.52	18.35	16.00	16.62	16.13	15.49	15.32	15.94	16.18	15.02	16.32±1.25	8.68±3.41

[1]Mean for 3 pigs.

[2]Mean and standard deviation for 36 pigs.

[3]Mean and standard deviation for 8 pigs.

[4]% Recovery = $\frac{\Sigma \text{ N in Ninhydrin-Positive Compounds (including } NH_3) \times 100}{\text{Total N Applied to Column}}$

Table 12. Apparent digestibility (%) of amino acids in the various rapeseed meal samples[1]

Amino Acids	Method of Processing and RSM Samples													
	Solvent				Prepress-solvent						Expeller		Mean[3]	Duncan's multiple range test[4]
	1	2	3	4	5	6	7	8	9	10	11	12		
Lysine	77.7[2]	69.0	73.1	70.6	71.2	69.9	73.7	75.0	72.1	72.2	67.6	68.5	71.6	ef
Histidine	87.7	78.9	82.6	83.5	79.5	83.4	84.0	83.4	79.9	74.5	82.1	84.7	82.0	b
Arginine	87.7	80.6	83.8	83.0	79.4	84.8	81.1	81.1	76.6	80.6	81.6	82.4	81.9	b
Aspartic acid	75.6	67.1	68.7	71.1	63.1	73.8	70.4	69.0	65.5	71.8	71.3	73.4	70.0	f
Threonine	75.2	66.4	72.0	72.2	66.2	73.1	72.9	71.4	66.4	70.4	64.8	75.8	70.6	f
Serine	77.0	71.7	74.7	77.3	70.7	75.4	75.6	73.4	68.0	72.8	71.4	79.5	74.0	cd
Glutamic acid	88.1	81.9	85.1	84.7	82.0	85.3	84.1	84.4	81.1	86.0	84.6	88.1	84.6	a
Proline	78.6	71.4	75.1	68.7	69.3	74.9	71.5	75.7	68.9	74.6	69.5	72.3	72.6	de
Glycine	78.8	69.3	73.5	73.8	68.3	74.0	74.5	74.3	66.1	73.9	67.8	75.6	72.4	de
Alanine	75.8	66.3	66.2	72.7	66.7	68.3	68.5	69.6	66.2	71.5	64.6	71.8	69.0	f
Valine	72.8	68.9	71.0	71.0	69.1	77.1	71.2	72.6	69.7	73.4	66.3	76.2	71.6	ef
Methionine	74.8	74.4	79.2	70.8	76.4	77.5	63.6	76.6	72.5	81.6	80.3	77.0	75.4	c
Isoleucine	73.2	67.9	72.2	71.9	63.9	75.2	72.2	73.2	69.4	71.7	68.7	71.7	70.9	ef
Leucine	76.3	71.3	76.8	75.0	70.1	77.3	75.9	77.0	64.2	74.1	72.7	79.0	74.1	cd
Tyrosine	75.2	67.0	63.0	71.9	64.9	75.7	69.1	73.6	66.0	72.1	70.2	76.4	72.6	de
Phenylalanine	72.7	69.8	73.9	73.5	67.6	75.9	75.7	74.0	70.6	71.2	70.2	76.4	72.6	de
Protein	78.9	71.5	74.6	74.6	70.2	75.8	74.6	76.5	71.5	73.7	74.2	76.4	74.4	

[1]Apparent digestibility calculated after amino acid analyses for the diets and feces were adjusted to 95% recovery (Knipfel et al., 1971).

[2]Mean of 3 determinations for each rapeseed meal sample.

[3]Overall mean for 36 determinations with the 12 RSM samples.

[4]Any mean not followed by same letter are significantly different ($P < 0.05$)

Table 13. True digestibility of amino acids of the various rapeseed meal samples[1].

Amino Acids	Method of Processing and RSM Samples													
	Solvent				Prepress-solvent						Expeller			Duncan's multiple range test[4]
	1	2	3	4	5	6	7	8	9	10	11	12	Mean[3]	
Lysine	80.6[2]	77.7	79.5	78.5	79.1	79.4	82.1	83.4	80.1	80.6	76.6	78.3	79.7	bcd
Histidine	89.2	84.0	87.7	87.2	84.1	88.8	88.4	87.9	85.0	82.6	86.9	85.9	86.5	a
Arginine	89.2	85.7	89.1	87.0	87.5	89.5	86.1	86.2	81.8	85.6	86.7	87.9	86.6	a
Aspartic acid	78.9	76.9	78.5	79.0	73.1	81.8	80.4	79.2	75.6	81.2	80.2	82.8	79.0	bcd
Threonine	78.1	75.4	80.1	79.7	74.4	80.7	80.9	80.0	75.1	79.0	73.6	83.3	78.4	cd
Serine	84.1	79.1	79.4	83.3	76.9	82.1	82.5	81.0	75.3	80.4	78.8	85.8	80.7	bc
Glutamic acid	89.5	86.4	89.2	88.4	86.2	89.8	88.3	88.8	86.2	90.1	89.1	91.6	88.6	a
Proline	80.3	76.5	80.5	74.3	74.4	80.3	76.2	80.5	74.1	80.0	74.7	77.7	77.4	d
Glycine	81.1	82.4	80.7	80.1	76.8	80.7	80.7	75.7	73.9	81.1	75.5	80.9	79.2	bcd
Alanine	78.9	75.7	76.3	80.8	75.8	77.9	77.8	79.4	75.4	82.2	74.2	81.2	78.0	d
Valine	78.6	77.3	79.5	78.4	76.8	82.8	79.8	81.0	77.8	81.4	74.6	83.7	79.4	bcd
Methionine	79.3	80.7	85.0	75.9	82.4	86.8	71.6	82.9	79.6	81.8	86.0	82.9	81.0	b
Isoleucine	78.8	76.4	80.0	78.6	72.6	82.5	79.9	81.3	77.4	79.9	77.1	79.2	78.7	cd
Leucine	81.1	78.4	82.6	81.0	77.2	83.9	82.2	83.9	73.4	81.4	80.0	85.5	80.9	bc
Tyrosine	80.9	76.2	74.3	78.8	74.1	83.4	77.4	82.2	75.8	78.9	76.3	80.6	78.2	d
Phenylalanine	78.2	77.2	81.5	79.8	75.5	83.0	82.4	83.4	78.2	79.3	82.4	83.3	80.2	bc
Protein	85.7	78.7	81.6	80.7	77.3	82.7	81.3	83.4	78.7	80.7	80.3	83.7	81.2	

[1]True digestibility calculated after amino acid analyses for the diets and feces were adjusted to 95% recovery (Knipfel et al., 1971).

[2]Mean of 3 determinations for each rapeseed meal sample.

[3]Overall mean for 36 determinations with the 12 RSM samples.

[4]Any mean not followed by same letter are significantly different ($P < 0.05$)

ibility of glutamic acid, arginine and histidine was found to be consistently ($P<0.05$) higher than that of the other amino acids. On the other hand true digestibility of tyrosine, alanine, aspartic acid, threonine and isoleucine tended to be lower than that of lysine, methionine, serine, leucine, phenylalanine, glycine and proline. The digestibility patterns for the individual amino acids tended to be similar whether the meals had been processed by the solvent, prepress-solvent or expeller method or whether they were comprised primarily of B. napus or B. campestris seeds.

Although the reasons for differences in the digestibility among the individual amino acids is not fully understood our results with RSM agree with those of other researchers. For example, Cho and Bayley (1970) found that the digestibility coefficients for glutamic acid were high whereas those of alanine, threonine, aspartic acid and tyrosine were low compared to the other amino acids for pigs fed RSM. Tao et al. (1971) reported similar results with chickens fed RSM. Olsen et al. (1968) also found that the true digestibility coefficients for glutamic acid, histidine and arginine were high whereas those for threonine, alanine and aspartic acid were low compared to other amino acids for rats fed bran, shorts and middlings. In general, these workers reported a relatively high digestibility for proline whereas proline digestibility ranked intermediate to low in the experiment herein described.

4.2 Experiment II. Comparison of the digestion and absorption of rapeseed meals with that of other protein sources.

4.2.1 Protein and dry matter digestibility.

Apparent and true protein digestibilities and dry matter digestibility based on a five day fecal collection period using Cr_2O_3 as index material are given in Table 14. Apparent and true digest-

Table 14. Apparent and true protein digestibilities (%) of rapeseed meal and other protein sources and dry matter digestibility of the diets by the growing pig[1].

Digestibility	Dietary Regimen							
					Rapeseed Meal			
	Casein	Autoclaved Casein	Soybean Isolate	Zein	RSM-1	RSM-4	RSM-5	Protein-Free
Apparent Protein Digestibility	91.8[2] ±1.6 a[3]	79.3 ±2.5 c	84.2 ±0.9 b	80.8 ±4.8 bc	75.5 ±2.5 cd	77.5 ±1.0 cd	74.7 ±1.6 d	-
True Protein Digestibility	99.5 ±2.6 a	85.0 ±3.0 cd	91.4 ±1.1 b	87.8 ±4.7 bc	82.4 ±3.0 d	84.5 ±2.1 cd	81.8 ±1.6 d	-
Dry Matter Digestibility	84.5 ±1.1 a	82.4 ±1.9 ab	83.2 ±2.3 a	82.7 ±1.9 ab	77.7 ±2.7 c	78.6 ±1.1 c	74.8 ±0.9 d	80.3[4] ±0.7 c

[1]Five-day fecal collection.

[2]All values mean±S.D.$_x$ for 4 pigs except as indicated by superscript 4.

[3]Means not followed by the same letter are significantly different ($P < 0.05$).

[4]Mean±S.D.$_x$ for 3 pigs.

ibilities of casein were significantly ($P<0.05$) higher than those of the other protein sources. Digestibility of soybean protein did not differ from that of zein but was significantly higher than the protein digestibilities of autoclaved casein and the three samples of rapeseed meal. There were no differences in protein digestibility among the rapeseed meal samples. However, the protein digestibility of RSM-5 was significantly ($P<0.05$) lower than that of the other protein sources although protein digestibility of RSM-1 and RSM-4 did not differ from that of autoclaved casein and zein. Dry matter digestibility of the rapeseed meal diets however was appreciably lower ($P<0.05$) than that of the other diets.

The results of the present study coincide with those of Cho and Bayley (1970), Oliver et al. (1970), Tao et al. (1971) and Flipot et al. (1971); namely that the protein and dry matter of rapeseed meal are poorly digested when compared to that of casein or soybean protein.

In an attempt to further explain the reason for the lower protein and dry matter digestibilities of RSM, piglets were sacrificed at the end of the digestibility trial and samples of digesta removed from different regions of the intestinal tract. The progress of digestion and absorption of protein and dry matter along the intestinal tract is summarized in Tables 15, 16, and 17. Protein digestibility coefficients for the caecum were similar to those for the rectum and feces with casein, autoclaved casein and the rapeseed meals. However, coefficients of digestibility for soybean protein were significantly higher ($P<0.05$) for the rectum and feces than for the caecum. With zein, digestibility coefficients for the rectal samples tended to be higher than those of caecum and feces. One factor that may have

Table 15. Apparent digestibility (%) of different proteins at various sites along the alimentary tract of pigs[1].

Source of Digesta	Protein Source						
					Rapeseed Meal		
	Casein	Autoclaved Casein	Soybean Isolate	Zein	RSM-1	RSM-4	RSM-5
Stomach	- 8.7[2] ±52.9	31.9 ± 4.0	4.6[2] ±33.6	21.3 ± 7.4	18.5 ±13.8	13.5 ± 6.3	14.9 ±10.2
	a[3]	a	a	a	a	a	a
Mid jejunum	71.3 ± 5.2	44.9 ±15.1	52.7 ± 1.5	26.1[2] ±19.8	37.1 ±23.4	60.4 ± 4.0	52.1 ±12.6
	a	bc	b	c	bc	ab	bc
Ileum	86.3[2] ± 4.5	78.2[2] ± 9.4	70.3[2] ± 3.5	60.8[2] ±17.3	70.4[2] ± 2.2	72.0[2] ± 4.8	77.2[2] ± 4.0
	a	ab	bc	c	bc	abc	ab
Caecum	88.4 ± 2.5	77.0 ± 1.4	74.7 ± 3.3	82.7 ± 3.4	73.4 ± 1.8	72.5 ± 3.9	73.5 ± 1.3
	a	c	cd	b	cd	d	cd
Rectum	91.1 ± 3.3	83.3[2] ± 1.4	84.1 ± 2.7	87.9 ± 1.1	75.2 ± 0.8	74.6[2] ± 1.3	76.3[2] ± 1.5
	a	c	c	b	d	d	d
Feces[4]	91.8 ± 1.6	79.3 ± 2.5	84.2 ± 0.9	80.8 ± 4.8	75.5 ± 2.5	77.5 ± 1.0	74.7 ± 1.6
	a	c	b	bc	cd	cd	d

[1]All values are mean ± $S.D._{\bar{x}}$ for 4 pigs, except as indicated by superscript 2.

[2]Mean ± S.D. for 3 pigs.

[3]For a given site along the intestinal tract means not followed by the same letter differ significantly ($P < 0.05$).

[4]Five-day fecal collection.

Table 16. True digestibility (%) of different proteins at various sites along the alimentary tract of pigs[1].

Source of Digesta	Protein Source						
					Rapeseed Meal		
	Casein	Autoclaved Casein	Soybean Isolate	Zein	RSM-1	RSM-4	RSM-5
Stomach	2.0[2] ±52.9	42.5 ± 8.2	30.9[2] ± 0.9	31.9 ±14.7	29.2 ±13.8	24.0 ±12.5	25.3 ±10.0
	a[3]	a	a	a	a	a	a
Mid jejunum	92.7 ± 5.2	66.0 ±14.9	74.2 ± 1.4	47.3[2] ±19.2	58.4 ±23.2	81.1 ± 3.9	73.4 ±12.9
	a	bc	b	c	bc	ab	bc
Ileum	97.0[2] ± 4.6	88.9[2] ± 8.7	81.1[2] ± 3.4	71.5[2] ±17.0	81.2[2] ± 2.3	83.8[2] ± 5.0	87.9[2] ± 4.4
	a	ab	bc	c	bc	abc	ab
Caecum	97.0 ± 2.4	85.6 ± 1.1	83.4 ± 2.6	91.2 ± 3.3	82.0 ± 1.9	80.9 ± 4.0	82.1 ± 1.3
	a	c	cd	b	cd	d	cd
Rectum	99.7 ± 2.5	92.2[2] ± 1.5	93.2 ± 2.8	96.8 ± 1.0	84.3 ± 0.8	83.3[2] ± 1.2	85.3[2] ± 1.4
	a	c	c	b	d	d	d
Feces[4]	99.5 ± 2.6	85.0 ± 3.0	91.4 ± 1.1	87.8 ± 4.7	82.4 ± 3.0	84.5 ± 2.1	81.8 ± 1.6
	a	cd	b	bc	d	cd	d

[1]All values are mean ± $S.D._x$ for 4 pigs except as indicated by superscript 2.

[2]Mean ± $S.D._x$ for 3 pigs.

[3]For a given site along the intestinal tract means not followed by the same letter differ significantly ($P < 0.05$).

[4]Five-day fecal collection.

Table 17. Dry matter digestibility (%) of different proteins at various sites along the alimentary tract of pigs[1].

Source of Digesta	Dietary Regimen							
					Rapeseed Meal			
	Casein	Autoclaved Casein	Soybean Isolate	Zein	RSM-1	RSM-4	RSM-5	Protein Free
Stomach	15.1[2] ±37.5	46.9 ±10.6	32.6[2] ±17.6	45.3 ±12.2	24.9 ±14.2	20.4 ± 6.6	25.3 ± 5.1	40.7[2] ±14.5
	a[3]	a	a	a	a	a	a	a
Mid jejunum	70.1 ± 5.0	66.2 ± 7.3	69.7 ± 4.5	60.2[2] ± 5.6	50.2 ±15.3	70.0 ± 4.0	62.4 ± 5.6	64.2[2] ±10.7
	a	a	a	a	a	a	a	a
Ileum	81.2 ± 5.5	84.2 ± 5.4	78.1 ± 1.68	80.1 ± 6.4	68.7 ± 5.0	68.1 ± 7.3	69.1 ± 2.1	72.1 ± 4.5
	a	a	ab	ab	c	c	c	bc
Caecum	81.0 ± 3.0	81.0 ± 1.6	77.8 ± 2.6	80.7 ± 1.5	73.1 ± 2.4	71.7 ± 2.4	71.7 ± 2.3	73.0 ± 2.3
	a	a	a	a	b	b	b	b
Rectum	86.1 ± 3.1	86.6[2] ± 0.4	85.2 ± 2.8	84.7 ± 0.8	76.3 ± 1.7	76.3[2] ± 1.9	75.3[2] ± 3.1	77.9[2] ± 0.7
	a	a	a	a	b	b	b	b
Feces[4]	84.5 ± 1.1	82.4 ± 1.9	83.2 ± 2.3	82.7 ± 1.9	77.7 ± 2.7	78.6 ± 1.1	74.8 ± 0.9	80.3[2] ± 0.7
	a	ab	a	ab	cd	c	d	bc

[1]All values except as indicated by superscript 2 are mean ± $S.D._x$ for 4 pigs.

[2]Mean ± $S.D._x$ for 3 pigs.

[3]For a given site along the intestinal tract means not followed by the same letter differ significantly ($P<0.05$).

[4]Five-day fecal collection.

contributed to the apparent discrepancy in digestibility coefficients between the samples from the caecum, rectum and feces was the fact that fecal samples represented a 5-day composite collection whereas samples from rectum and caecum were single casual samples collected at the time of slaughter.

Apparent and true protein digestibility coefficients on the basis of digesta obtained from the ileum also were similar to those for caecum, rectum and feces for all proteins sources except soybean and zein. The digestibility coefficient for soybean protein was significantly lower ($P<0.05$) for the ileum than the rectum or feces. Digestibility coefficients for zein also tended to increase from the ileum to the rectum but there was appreciable variation in digestibility coefficients among pigs fed zein. The higher digestibility of soybean in the rectum and feces compared to the ileum and caecum coincides with the suggestion of Cho and Bayley (1972) that fermentation of protein might be responsible for the improvement in apparent digestibility of this protein. Although such an explanation may be satisfactory for soybean it does not appear to apply to any of the other protein sources used in the present study.

Protein digestibility coefficients in the mid jejunum were highly variable both within and among protein sources. Nevertheless, digestibility of casein in the mid jejunum was significantly ($P<0.05$) higher than for the other protein sources except for RSM-4. The rapid rate of digestion and absorption of casein probably explains the significantly ($P<0.05$) higher digestibility coefficients for this protein in the lower regions of the gut and in the feces. However, except for casein, there appears to be little relationship between the

rate of digestion and absorption of the various proteins sources in the proximal small intestine and their eventual digestibility. The present results do not indicate that the initial rate of digestion of rapeseed meal is depressed compared to the other proteins sources. The apparent digestibility on the basis of digesta samples from the mid jejunum ranged from 37.1 to 60.4% for the rapeseed meals compared to 26.1, 44.9 and 52.7% for zein, autoclaved casein and soybean protein, respectively. Part of the apparent discrepancies between protein digestibilities based on digesta samples from the mid jejunum and the eventual digestibility of the various proteins sources based on feces may be due to differential movement of digesta and index material in various regions of the alimentary tract as indicated by the appreciable disappearance of dry matter (Table 17) and protein in the stomach.. In addition, fermentation of non-digested protein may contribute to the poor relationship between digestibility coefficients in ileum and feces for soybean. However, neither of these suggestions is satisfactory as an explanation of the lower digestibility of rapeseed protein.

4.2.2 Total protein, soluble protein and amino nitrogen in digesta and feces.

Progress of digestion for the various protein sources also was studied by determining the content of total protein, soluble protein and amino nitrogen in digesta obtained from different sites along the alimentary tract (Table 18). There were no marked differences in the percent of total protein, soluble protein and amino nitrogen in the stomach contents of pigs fed the different protein sources. Total protein content of digesta from the mid jejunum of pigs fed rapeseed meals was similar to that of stomach contents whereas the level of protein tended to decrease for pigs fed casein and increase for those

Table 18. Total protein, soluble protein and amino nitrogen in digesta from pigs fed rapeseed meal and other protein sources[1].

Source of Digesta	Nitrogen Fraction	Dietary Regimen							
						Rapeseed Meal			
		Casein	Autoclaved Casein	Soybean Isolate	Zein	RSM-1	RSM-4	RSM-5	Protein Free
Diet	Total Protein[2]	15.67	15.70	15.50	15.53	15.66	15.69	16.02	0.42
Stomach	Total Protein[3]	19.7[4] ± 1.8	20.7[5] ± 1.6	21.7[4] ± 1.9	23.1 ± 3.2	17.7 ± 0.9	17.5 ± 2.0	18.1 ± 1.4	2.9[4] ± 0.6
	Soluble Protein[6]	46.1 ±19.7	33.1 ± 6.6	44.2 ±10.6	26.6 ±11.1	45.6 ± 7.1	39.8 ± 6.3	42.0 ± 5.0	75.0 ± 1.9
	Amino Nitrogen[7]	8.5 ± 3.0	9.7 ± 1.1	7.6 ± 2.8	8.8 ± 1.4	8.2 ± 2.5	6.5 ± 2.4	8.1 ± 3.2	1.5 ± 0.6
Mid jejunum	Total Protein	15.3 ± 2.8	26.0 ± 4.4	24.7 ± 3.3	29.5[4] ± 7.7	19.7 ± 2.2	21.4 ± 3.2	19.9 ± 2.4	9.8[4] ± 2.7
	Soluble Protein	89.7 ± 2.3	90.2 ± 3.7	88.2 ± 2.6	72.3 ± 5.5	65.7 ± 4.3	66.4 ± 8.4	60.2 ± 9.6	88.7 ± 6.2
	Amino Nitrogen	34.1 ± 8.8	21.0 ± 4.3	19.8 ± 2.9	32.3 ± 6.5	24.3 ± 4.8	23.9 ±11.9	27.3 ± 8.9	13.3 ± 2.0
Ileum	Total Protein	11.5[4] ± 1.3	21.7[4] ± 1.9	22.6[4] ± 4.1	27.1[4] ± 8.0	15.1[4] ± 4.3	13.6[4] ± 0.5	11.7[4] ± 3.5	6.2[4] ± 1.7
	Soluble Protein	83.9 ± 2.5	87.8 ± 4.5	88.5 ± 3.2	71.0 ± 5.8	63.5 ±11.8	56.2 ± 1.0	63.4 ± 3.5	75.3 ±11.5
	Amino Nitrogen	29.0 ± 8.6	16.4 ± 5.0	15.2 ± 1.4	37.2 ± 2.2	18.8 ± 4.1	19.5 ± 5.3	19.1 ± 4.1	18.0 ± 3.8
Caecum	Total Protein	9.7 ± 2.5	19.1 ± 1.1	17.8 ± 0.6	14.3 ± 2.5	15.7 ± 2.0	15.7 ± 1.4	15.0 ± 2.0	5.0[4] ± 0.2
	Soluble Protein	45.3 ± 3.2	74.6 ± 5.5	66.6 ± 7.0	30.7 ± 4.7	38.5 ± 4.9	31.7 ± 3.5	33.7 ± 6.8	71.5 ± 8.7
	Amino Nitrogen	25.8 ±10.2	21.4 ± 4.7	14.9 ± 3.9	35.4 ±10.4	25.1 ± 4.2	23.7 ± 6.2	23.6 ± 6.9	18.4 ± 2.9
Rectum	Total Protein	10.0 ± 3.1	20.0 ± 1.4	16.9 ± 1.5	12.9 ± 1.5	16.5 ± 1.0	17.5 ± 1.4	15.4 ± 1.3	6.4[4] ± 1.0
Feces	Total Protein	8.5[6] ± 0.8	18.8 ± 2.3	14.8 ± 1.6	17.4 ± 2.7	17.5 ± 0.8	16.9 ± 1.4	15.9 ± 0.9	5.7[4] ± 0.4
	Soluble Protein	29.1 ± 3.1	45.5 ±10.5	50.6 ± 8.3	17.2 ± 4.8	23.1 ± 3.3	18.8 ± 3.2	20.1 ± 4.4	31.1 ± 7.3

[1]Statistical analysis of the data is given in Appendix Table I.

[2]Expressed as % of dry matter content of diet.

[3]Expressed as % of dry matter content of digesta.

[4]All values are mean ± $S.D._x$ for 3 pigs.

[5]Mean ± $S.D._x$ for 4 pigs except as indicated by superscript 4.

[6]Expressed as % of total protein of digesta.

[7]Amino nitrogen determined by formal titration and expressed as % of soluble protein using glycine as a reference.

fed autoclaved casein, soybean protein and zein. The slightly lower protein level in digesta from the mid jejunum of pigs fed casein compared to that of the other protein sources coincides with the rapid absorption found for this protein (Table 15). On the other hand there was an appreciable increase for all groups in the proportion of water-soluble protein in digesta from the mid jejunum compared to that from the stomach. However, the proportion of soluble protein in digesta from the mid jejunum and ileum was significantly ($P < 0.05$) lower for pigs fed zein and rapeseed meals (60 to 72% of the total protein) than for those fed casein, autoclaved casein and soybean protein (84 to 92% of the total protein). The increase in soluble protein was probably the result of hydrolysis of the proteins as evidenced by the concomitant increase in amino N. The proportion of amino N in the soluble protein fraction from the mid jejunum and ileum tended to be higher for pigs fed casein and zein than for those fed the other protein sources. However, there appears to be little relationship between the content of amino N in the mid jejunum and ileum and the eventual digestibility of the various proteins. For example zein was relatively poorly absorbed compared to casein although the proportion of amino N was similar in digesta from the mid jejunum and ileum of pigs fed both these proteins.

Total protein content did not change appreciably from the ileum to the lower regions of the digestive tract for pigs fed casein, autoclaved casein and rapeseed meals. However, the proportion of total protein in digesta decreased from the ileum to the feces for pigs fed the soybean and zein diets. This decrease in protein content with soybean coincides with the improvement in protein digestibility for this diet. Whether fermentation of protein or some other factors are

responsible for the improvement in protein digestibility for soybean protein and zein is not resolved in this experiment. The fact that there were no differences in the total protein content or marked improvement in protein digestibility coefficients among digesta samples taken from the lower regions of the intestinal tract of pigs fed the other protein sources tends to rule out the possibility of extensive fermentation of protein in the caecum and colon.

The relatively high proportion of amino N in the soluble fraction of digesta from the mid jejunum, ileum and caecum of pigs fed zein indicates that once solubilized zein is readily hydrolyzed. Chen et al. (1962) also found that the proportion of amino N in the TCA soluble fraction of digesta from the small intestine of rats fed zein was relatively high compared to that of rats fed gelatin although there was a greater proportion of insoluble protein with the zein diet.

No clear pattern can be drawn on the digestion of rapeseed meal from the data herein reported. The results suggest that the digestion of rapeseed meal is limited by the relatively low solubility of this protein in the small intestine. Thus although the initial digestion and absorption of RSM in the mid jejunum did not differ from that of other proteins, hydrolysis in the lower regions of the alimentary tract tended to be lower for rapeseed meal than for the other proteins. However, it must be born in mind that soluble protein and amino nitrogen determinations were made on the residue that remained in the digesta. By the time digesta had reached the ileum, for example, more than 70% of the protein had been absorbed irrespective of dietary protein source. Digestibility coefficients on the basis of digesta from the ileum were similar for all protein sources except casein. In feces, by contrast,

the apparent and true digestibility of soybean was significantly ($P<0.05$) higher than that of autoclaved casein and the RSMs although the digestibility of zein did not differ ($P<0.05$) from that of soybean protein. This improvement in protein digestibility for soybean protein in the lower region of the intestinal tract is in agreement with the evidence given by various researchers (Nesheim and Carpenter, 1967; Payne et al., 1968; Salter and Coates, 1971; Cho and Bayley, 1972) that microbial action on undigested protein in the caecum and colon improves the apparent digestibility of the proteins. However, the present experiment does not resolve whether the apparent improvement in digestibility of soybean protein, presumably as a result of fermentation is related to solubility of the residual protein entering the large intestine or other factors. A much higher ($P<0.05$) proportion of soluble protein entered the large intestine of pigs fed autoclaved casein and soybean protein than for those fed rapeseed meal. It is possible that the extent of fermentation of residual digesta from autoclaved casein may have been less than that of soybean protein because of known effects of the Maillard reaction on lysine availability and protein digestibility. Unfortunately there is no clear indication that low solubility and/or apparent lack of fermentation are factors in the generally lower protein digestibility of rapeseed meal compared to isolated soybean protein.

4.2.3 Amino acid pattern in diet and digesta

Some distinct differences existed in the amino acid composition of protein used in this experiment (Table 19). Zein for example was almost devoid of lysine (0.24 gm/16 gm N) and relatively low in histidine arginine and glycine compared to the other proteins. On the other hand casein was almost devoid of cystine and relatively low in alanine

Table 19. Amino acid composition of diets used in Experiment II (gm/16 gm N).

Amino Acid	Protein Source						
					Rapeseed Meal		
	Casein	Heated[1] Casein	Isolated[2] Soy	Zein	RSM-1[3]	RSM-4[4]	RSM-5[5]
Lysine	7.65[6]	7.69	5.88	0.24	5.51	5.37	5.24
Histidine	2.68	2.49	2.65	1.25	2.51	3.03	2.70
Arginine	3.75	3.37	8.41	1.55	5.96	6.61	6.48
Aspartic Acid	7.55	7.18	12.44	5.77	7.73	7.63	7.87
Threonine	4.51	4.34	3.44	2.93	4.72	4.51	4.83
Serine	6.08	5.85	5.22	5.78	4.86	4.86	4.82
Glutamic Acid	23.53	23.69	20.00	25.10	19.83	19.07	19.17
Proline	11.25	11.13	5.42	9.40	7.18	6.03	6.03
Glycine	2.10	2.03	4.07	1.27	5.55	5.40	5.56
Alanine	3.38	3.26	4.09	9.95	4.66	4.94	5.12
Cystine	0.08	0.15	-	0.20	2.24	2.54	1.59
Valine	6.42	6.47	4.54	3.58	5.14	5.05	5.23
Methionine	2.84	2.72	1.14	1.08	1.87	1.94	2.05
Isoleucine	4.91	5.02	4.13	3.76	3.91	4.12	3.67
Leucine	10.14	9.76	8.26	21.49	7.64	7.90	7.63
Tyrosine	4.76	4.68	3.65	4.32	2.45	2.95	3.04
Phenylalanine	5.29	5.18	5.39	7.18	3.92	4.20	4.07

[1]Autoclaved at 15 psi for 12 hr.

[2]Alpha protein, Nutritional Biochemicals Co., Cleveland, Ohio.

[3]B. napus cv. Oro. All solvent processed.

[4]B. napus cv. Bronowski. All solvent processed.

[5]B. campestris. Prepress-solvent processed.

[6]Amino acid values were corrected to 95% recovery (Knipfel et al., 1971).

whereas isolated soybean protein was low in methionine and proline. Casein tended to be higher in lysine and methionine whereas isolated soybean protein was high in arginine and aspartic acid and zein contained a high level of alanine and leucine. Rapeseed meal contained intermediate levels of essential and non-essential amino acids although the level of cystine was relatively high in the RSM (1.59 to 2.54 gm/16 gm of N) compared to the other proteins.

Although browning of the casein occurred as the result of autoclaving with 2% glucose there was no marked destruction of amino acids. In fact the lysine content of the autoclaved casein was identical to that of the non-heated casein (7.69 and 7.65 gm/16 gm N), respectively. It is possible that the failure to find extensive destruction of lysine in our experiment was due to the low level of glucose added relative to the amount of lysine in casein. Calculations show that no more than 23% of the total lysine would be involved in a "Maillard type" reaction if there was complete reaction with the 2% added glucose.

Comparison of the amino acid composition of diet, digesta and feces for the proteins under investigation in the present study revealed that, in general, amino acid composition of digesta and feces reflected the amino acid composition of the diet (Table 20). For example the unusual low amount of lysine, histidine and arginine in digesta of pigs fed zein is a reflection of their low concentration in this protein. Similarly the high level of leucine and alanine in zein resulted in significantly ($P<0.01$) higher amounts of these amino acids in the digesta and feces of pigs fed this protein. Even the slightly higher content of lysine in casein appears to result in a higher lysine content in the digesta and feces. Yet there were some rather marked exceptions to

Table 20. Amino acid composition of the diet, digesta and feces when rapeseed and other protein sources supplied the sole source of dietary protein.

Dietary Regimen	Sample	Amino Acid Composition (gm/16 gm N)[1]																% N Recovery[3]	Protein (%)
		Lys	His	Arg	Asp	Thr	Ser	Glu	Prol	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe		
Casein	Diet	7.6	2.7	3.8	7.6	4.5	6.1	23.5	11.2	2.1	3.4	6.4	2.8	4.9	10.1	4.8	5.3	97.6	15.7
	Mid jejunum	6.6[2]	1.7	3.3	8.4	4.9	5.4	16.3	12.4	16.5	3.6	4.0	1.1	3.3	4.8	2.0	2.6	91.9±4.7[3]	15.5±3.4
	Ileum	6.1	1.4	3.5	7.5	6.4	5.5	18.7	7.1	14.2	4.3	4.4	1.4	3.0	4.9	2.7	3.3	77.5±1.6	11.5±1.3
	Caecum	6.5	1.5	4.0	10.4	5.1	4.4	12.6	4.6	5.2	6.5	4.6	2.4	4.0	6.6	3.5	5.2	75.7±1.7	10.1±2.9
	Feces	7.1	1.4	4.1	10.8	5.3	4.6	12.6	5.3	5.4	6.0	5.4	2.5	4.3	7.5	3.9	4.2	75.6±1.9	8.4±1.0
Autoclaved Casein	Diet	7.7	2.5	3.4	7.2	4.3	5.8	23.7	11.1	2.0	3.3	6.5	2.7	5.0	9.8	4.7	5.2	96.5	15.7
	Mid jejunum	6.7	2.7	2.8	9.6	4.6	5.6	24.6	12.2	9.0	3.6	5.0	2.0	4.4	6.8	2.7	3.1	92.2±4.6	27.9±2.7
	Ileum	8.8	2.0	2.1	14.5	5.4	6.0	25.7	11.2	6.3	3.5	4.9	2.9	5.2	5.5	1.6	1.9	80.7±3.3	21.7±1.3
	Caecum	9.5	1.8	1.7	13.0	4.8	5.0	22.3	8.7	3.4	4.4	5.2	2.7	5.6	5.9	2.1	2.6	74.2±1.4	19.5±0.8
	Feces	7.9	2.1	3.2	10.8	4.8	5.2	16.6	6.3	3.6	4.8	5.7	2.7	5.1	6.6	2.8	3.2	75.3±2.6	18.5±2.7
Soybean Isolate	Diet	5.9	2.6	8.4	12.4	3.4	5.2	20.0	5.4	4.7	4.1	4.5	1.1	4.1	8.3	3.6	5.4	97.5	15.5
	Mid jejunum	4.9	1.9	4.8	14.3	4.3	6.9	19.2	8.9	9.8	4.1	3.8	1.3	3.2	5.3	2.5	3.2	94.4±3.5	26.1±2.4
	Ileum	5.6	2.2	4.4	15.5	4.9	7.3	17.2	8.5	8.6	3.3	3.2	1.5	2.9	5.5	2.0	3.2	80.0±4.4	21.4±4.1
	Caecum	5.7	1.8	4.1	14.0	5.2	6.9	16.5	4.8	5.3	4.8	4.3	1.6	4.1	6.0	2.6	4.2	77.5±6.1	17.8±0.6
	Feces	5.6	1.8	3.9	12.3	5.6	6.6	14.1	4.6	5.0	5.8	5.0	2.4	3.9	6.3	3.1	4.0	75.8±1.3	15.4±1.4
Zein	Diet	0.2	1.2	1.6	5.8	2.9	5.8	25.1	9.4	1.3	10.0	3.6	1.1	3.8	21.5	4.3	7.2	96.6	15.5
	Mid jejunum	1.8	1.1	2.5	6.4	3.7	5.1	20.8	9.4	10.4	7.7	3.8	1.0	3.6	13.6	3.7	4.9	94.1±1.4	29.5±7.7
	Ileum	1.3	1.6	2.1	5.5	3.6	4.8	22.3	11.8	4.2	9.1	3.6	1.3	3.9	15.4	4.0	5.9	82.8±3.6	30.6±4.7
	Caecum	2.5	1.3	1.8	6.5	3.4	4.5	19.8	7.9	3.0	8.6	4.2	1.5	4.1	14.7	3.5	6.0	77.8±3.2	15.1±2.5
	Feces	2.8	1.4	2.4	8.0	4.2	5.3	19.3	5.7	3.1	8.5	4.3	1.9	4.2	15.1	3.8	5.0	77.8±2.6	18.7±1.0
RSM-1	Diet	5.5	2.5	6.0	7.7	4.7	4.9	19.8	7.2	5.6	4.7	5.1	1.9	3.9	7.6	2.4	3.9	87.7	15.7
	Mid jejunum	4.6	2.1	4.3	6.8	4.6	4.8	15.5	10.0	12.8	4.3	4.4	0.8	3.0	5.6	2.0	2.8	82.1±6.6	19.5±2.6
	Ileum	6.6	1.9	4.2	8.6	5.9	5.4	12.7	10.6	7.5	4.4	5.3	1.1	3.2	6.1	2.8	3.4	68.1±4.8	15.1±2.4
	Caecum	6.7	1.9	4.3	9.5	5.7	5.0	12.1	7.0	5.7	6.2	5.6	1.8	4.3	7.4	3.3	4.7	68.2±5.1	16.5±1.5
	Feces	6.6	1.9	4.1	9.4	5.5	4.9	11.6	6.0	5.8	6.1	6.1	2.4	4.5	7.5	3.3	4.1	71.2±0.8	17.7±0.9
RSM-5	Diet	5.2	2.7	6.5	7.9	4.8	4.8	19.2	7.1	5.6	5.1	5.2	2.0	3.7	7.6	3.0	4.1	86.0	16.0
	Mid jejunum	6.0	2.1	4.5	8.7	5.3	5.1	14.5	8.8	9.8	4.5	4.7	1.2	3.3	6.5	2.4	3.5	81.4±8.4	19.9±2.5
	Ileum	6.4	1.9	3.3	9.0	6.1	5.1	12.0	10.6	7.8	4.5	5.1	1.3	3.6	6.7	2.5	3.6	67.0±3.1	11.7±2.8
	Caecum	7.0	1.7	3.6	10.0	5.8	5.3	12.1	7.4	6.1	5.8	6.0	1.9	4.6	8.0	3.3	4.8	68.2±4.0	15.2±2.3
	Feces	6.2	1.7	4.2	9.5	5.4	4.7	11.2	5.7	5.5	5.8	5.4	2.3	4.3	7.4	3.0	4.0	68.9±3.4	15.5±0.7
Protein-Free	Mid jejunum	2.6	1.0	4.1	5.1	3.5	3.8	6.1	26.1	21.2	3.8	3.1	0.6	1.5	3.4	1.6	1.7	94.0±3.4	9.8±2.7
	Ileum	2.6	1.5	3.6	6.4	5.2	4.7	8.1	28.6	16.7	3.8	3.3	0.7	1.9	3.9	1.7	2.0	79.8±3.4	6.2±1.7
	Caecum	6.3	1.7	4.2	9.9	5.6	4.8	11.8	10.5	7.6	6.0	5.1	2.3	3.9	6.9	3.0	3.9	76.0±1.8	5.0±0.2
	Feces	6.9	1.9	4.3	10.5	5.9	5.2	12.0	3.5	5.2	7.0	5.6	2.7	4.8	7.4	3.3	4.0	74.7±2.2	5.7±0.5

[1] Amino acid values were corrected to 95% recovery (Knipfel et al., 1971).

[2] Each value for digesta from the mid jejunum, ileum, caecum and feces represents the mean (± $S.D._{\bar{x}}$) for 3 observations. Individual values in each region of the intestinal tract and the feces were from the same pigs.

[3] % Recovery = $\frac{\Sigma \text{ N in Ninhydrin-Positive Compounds (including } NH_3) \times 100}{\text{Total N Applied to Column}}$

this general pattern. High levels of glycine and proline in endogenous protein (see digesta from the mid jejunum and ileum for pigs fed the protein-free diet - Table 20) resulted in elevated levels of these amino acids in the digesta of most groups. The amino acid patterns in digesta from the mid jejunum and ileum were fairly similar for all protein sources. By contrast there was a marked difference in the amino acid composition of digesta from the ileum and caecum although the influence of the dietary protein was still noticeable. The major change was a decrease in the proportion of proline and glycine. In general there was an increase in the proportion of alanine, isoleucine, leucine, tyrosine and phenylalanine except when the content of these amino acids in the diet was relatively high as in the case of alanine and leucine with zein. There was an appreciably higher content of lysine in caecal contents and in feces than in digesta from the ileum for pigs fed the zein and protein-free diets whereas the lysine content remained relatively constant with the other proteins. The increase in lysine between the ileum and the feces with the zein diet may be related to the low level of lysine in zein. As a consequence the contribution of endogenous lysine would be more pronounced with zein than with the other protein sources. There was a substantial ($P < 0.01$) increase in the methionine content in the digesta from the ileum to the feces for pigs fed the protein-free diet. The methionine content also increased between the ileum and feces for all protein sources except autoclaved casein. On the other hand no appreciable changes occurred in the

proportion of histidine, arginine, aspartic acid and threonine in the digesta from the mid jejunum, ileum and caecum.

Comparison of the amino acid composition of digesta from the caecum with that of the feces revealed that little change occurred in the colon with any of the protein sources except for a decrease in proline and glycine and a slight increase in methionine. The fecal amino acid pattern for casein was very similar to that of the fecal pattern for the protein-free regimen. Thus the influence of metabolic amino acids on the fecal amino acid patterns appears to be most pronounced when the dietary protein is limiting in a particular amino acid or when the dietary protein is readily digested and absorbed. In general, however, fecal amino acid pattern tended to reflect the amino acid composition of the diet.

The results of this experiment tend to rule out the possibility of extensive dilution of exogenous proteins by endogenous proteins as suggested by Nasset and co-workers (1955, 1957, 1961, 1963). Rather, the present observations support those of Crompton and Nesheim (1969) and Carlson and Bayley (1970) that the dilution of digesta and feces by endogenous protein is insufficient to mask the amino acid pattern of dietary proteins. Although the present observations do not support the postulation of extensive fermentation in the colon, except in the case of soybean protein where there was a substantial decrease in protein content (Table 18), the present results agree with those of Cho and Bayley (1972) in that major changes occurred in the content of proline, glycine, methionine and to a lesser extent alanine and leucine, between the ileum and feces.

4.2.4 Amino acid digestibility in feces and digesta

Apparent digestibilities of the individual amino acids followed a similar pattern to that of the total protein (Table 21). In general, coefficients of amino acid digestibility were higher for casein than for the other protein sources. Similarly amino acid digestibilities tended to be lower for rapeseed meal. Autoclaving casein depressed the apparent digestibility of all amino acids but the effect of heat damage was most noticeable for aspartic acid, glycine and alanine.

Amino acid composition of the dietary proteins (Table 19) had an appreciable effect on the apparent digestibilities of the individual amino acids. A relatively low content of an amino acid resulted in lower apparent digestibility for this amino acid. For example the digestibility coefficient for lysine was negative with zein (Table 21). The lower apparent digestibilities of arginine, glycine and methionine with zein and methionine with soybean protein also corresponded to lower levels of these amino acids in zein and soybean protein relative to their levels in the other proteins. Thus, some of the differences in apparent availabilities of amino acids among proteins may be the result of differences in the amino acid content of the dietary proteins rather than true differences in the availability of the amino acids. In proteins where one or more amino acids are limiting the contribution of metabolic amino acids to the feces would be appreciable relative to that of dietary origin. For example, the true digestibilities (Table 22) of glycine for the different proteins ranged from 82.5 to 97.5 whereas the apparent digestibility coefficients varied from 47.5 to 80.6. Similarly true digestibility coefficients for the amino acids in casein ranged from 97.5 to 100.3 compared to apparent digestibility coefficients of 79.9 to 95.6.

Table 21. Apparent digestibility (%) of the individual amino acids of rapeseed meal and other protein sources[1].

Amino Acids	Protein Source					
					Rapeseed Meal	
	Casein	Autoclaved Casein	Soybean Isolate	Zein	RSM-1	RSM-5
Lysine	92.6 a[2] CD[3]	77.2 c EF	84.6 b CD	-160.1 e G	70.4 d EF	69.6 d E
Histidine	95.3 a AB	83.1 cd ABCDE	89.2 b B	77.5 e CD	80.5 d ABC	86.0 bc A
Arginine	90.8 a DE	81.0 b CDFF	92.4 a A	67.0 c E	82.2 b AB	82.8 b AB
Aspartic acid	88.2 a D	68.4 b G	84.3 a CD	72.0 b DE	70.1 b EF	70.0 b DE
Threonine	90.2 a E	76.3 b F	73.0 bc F	71.6 c DE	71.4 c EF	72.0 c CDE
Serine	93.7 a BC	81.8 b BCDEF	81.2 b E	82.2 b ABC	75.2 c CDE	75.7 c C
Glutamic acid	95.6 a AB	85.2 b ABCD	88.8 b B	85.6 b AB	85.8 b A	85.6 b A
Proline	96.0 a A	88.3 b A	86.7 b BC	88.3 b A	80.0 c BCD	79.6 c B
Glycine	79.9 a F	63.5 b G	80.6 a E	47.5 c F	74.6 a DE	75.1 a C
Alanine	85.0 a E	68.1 d G	77.5 bc F	83.7 ab ABC	68.2 d F	71.8 cd CDE
Valine	93.0 a C	81.8 b BCDEF	82.9 b DE	76.6 c CD	71.2 d EF	74.2 cd CD
Methionine	92.7 a CD	79.1 b DEF	66.5 d G	64.6 d E	62.0 d G	72.1 c CDE
Isoleucine	92.7 a CD	78.5 c EF	84.8 b CD	78.0 c BCD	71.5 d EF	69.9 d DE
Leucine	93.9 a ABC	85.2 b ABC	88.2 b B	86.9 b A	75.8 c CDE	74.8 c C
Tyrosine	93.4 a BC	86.9 b AB	86.2 b BC	83.1 b ABC	66.2 c FG	74.5 c C
Phenylalanine	93.6 a BC	85.6 b ABC	88.1 b B	86.2 b A	75.3 c CDE	73.5 c CDE
Protein	91.6 a	79.3 c	84.1 b	80.8 bc	75.3 cd	74.7 d

[1]Each value represents the mean of 4 observations based on fecal samples.

[2]Values in the same row followed by the same small letter did not differ significantly ($P < 0.05$).

[3]Values in the same column followed by the same capital letter did not differ statistically ($P < 0.05$).

Table 22. True digestibility (%) of the individual amino acids of rapeseed meal and other protein sources[1].

Amino Acids	Protein Source					
	Casein	Autoclaved Casein	Soybean Isolate	Zein	Rapeseed Meal	
					RSM-1	RSM-5
Lysine	99.1 a[2] A[3]	84.6 c DE	93.1 b BC	51.4 e A	79.4 d DEF	79.0 d C
Histidine	100.3 a A	88.5 cd ABCD	94.3 b AB	88.5 cd B	85.9 d ABC	90.7 c A
Arginine	99.0 a A	89.8 b ABCD	96.1 a A	87.1 b B	87.4 b AB	87.5 b ABC
Aspartic acid	98.2 a A	78.9 d FG	90.4 b CD	85.5 c B	79.6 d DEF	80.8 cd C
Threonine	99.6 a A	85.8 b BCDE	85.5 b FG	86.5 b B	80.3 c DE	79.8 c C
Serine	100.1 a A	88.5 b ABCDE	87.5 b EF	89.3 b B	83.2 c BCD	83.1 c BC
Glutamic acid	99.3 a A	88.8 c ABCDE	93.2 b AB	89.2 c B	90.2 bc A	89.5 c AB
Proline	98.2 a A	90.6 b ABC	91.4 b BCD	91.0 b B	83.5 c BCD	82.6 c BC
Glycine	97.5 a A	77.6 c G	89.7 b DE	82.5 c B	81.2 c CDE	80.9 c C
Alanine	99.8 a A	83.4 bc EF	89.8 b DE	89.0 b B	78.9 c DEF	80.5 c C
Valine	99.3 a A	88.1 bc ABCDE	92.0 b BCD	87.6 c B	79.1 d DEF	81.1 d C
Methionine	99.4 a A	85.7 b BCDE	83.1 b G	82.6 b B	74.4 c F	83.9 b BC
Isoleucine	99.6 a A	85.3 c CDE	93.1 b BC	87.3 c B	80.3 d DE	79.2 d C
Leucine	99.2 a A	90.7 bc ABC	94.2 b AB	89.5 c B	82.5 d BCD	81.2 d C
Tyrosine	98.3 a A	91.9 bc A	93.0 b BC	88.8 c B	75.9 d EF	82.3 d C
Phenylalanine	98.7 a A	91.1 b AB	93.0 b BC	90.3 b B	79.9 c DEF	81.1 c C
Protein	99.4 a	86.4 c	91.4 b	88.0 bc	82.4 d	81.8 d

[1]Each value represents the mean of 4 observations based on fecal samples.

[2]Values in the same row followed by the same small letter did not differ significantly ($P < 0.05$).

[3]Values in the same column followed by the same capital letter did not differ statistically ($P < 0.05$).

No general pattern was observed in the true digestibilities of the individual amino acids within the different protein sources. There were no differences in the true digestibility coefficients among the 16 amino acids of casein. Similarly with the exception of lysine, true digestibility coefficients were similar for all amino acids in zein. On the other hand, within soybean, digestibilities of histidine, arginine, tyrosine, phenylalanine, glutamic acid and leucine were significantly higher ($P<0.05$) that those of methionine, threonine and serine. Autoclaving casein reduced the availability of all amino acids but the degree of reduction varied among amino acids. The true availability of tyrosine and phenylalanine was less affected by heat treatment than aspartic acid and glycine whereas the true availabilities of the other amino acids, including lysine, followed that of the protein. Valle-Riesta and Barnes (1970) and Boctor and Harper (1968) also reported that heat treatment reduced the nutritional availability of lysine to the same degree as total dietary nitrogen. No particular reason can be given to explain the lower availability of aspartic acid and glycine although *in vitro* studies (Evans and Butts, 1949; Ford, 1965; Ford and Salter, 1966) have indicated that heat treatment affects the release of dicarboxylic amino acids such as aspartic acid and glutamic acid, as well as the release of lysine.

Furthermore, digestibility coefficients for the amino acids in casein were significantly ($P<0.05$) higher than those for the other protein sources, except for arginine in soybean protein. These observations coincide with those of Carlson and Bayley (1970) and Flipot *et al*. (1971) who also reported high digestibility coefficients for all amino acids in casein. In general, the availabilities of the amino acids in soybean protein were higher than those for proteins other than

casein although the availabilities of threonine, serine, proline and methionine in soybean protein did not differ from those of autoclaved casein and zein. Coefficients of digestibility for the amino acids in the rapeseed meal were significantly ($P < 0.05$) lower than those for the other proteins except for histidine, arginine, glutamic acid, glycine, alanine and methionine.

The progress of apparent and true amino acid absorption along the intestinal tract is shown in Tables 23 and 24. Digestibility coefficients for the individual amino acids in each protein source did not differ appreciably for the ileum, caecum and feces except for pigs fed soybean protein and zein. With the latter two proteins improvement in apparent digestibility of the individual amino acids between the ileum and caecum coincided with the appreciable improvement found in protein digestibility. However there were some marked exceptions to this general pattern. The apparent digestibility of methionine tended to decrease from the ileum to the feces of pigs fed rapeseed meal but remained fairly constant for the other protein sources. Furthermore with the exception of RSM-5, there was a substantial improvement in the apparent digestibility of glycine between the ileum and caecum. This improvement in the apparent digestibility of glycine was probably related to the high level of glycine in the endogenous protein and the marked decrease in glycine between the ileum and caecum for pigs fed the protein-free diet (Table 20). Nixon and Mawer (1970[a]) also reported high levels of glycine in digesta from the small intestine of humans fed casein and gelatin. They suggested that the high level of glycine was associated with bile salts. The lower level of glycine in the digesta from the

Table 23. Apparent digestibility[1] (%) of the individual amino acids of rapeseed meal and other protein sources at various sites along the intestinal tract of growing pigs.

Dietary Regimen	Sample	Amino Acids															Protein Dig.(%) ± S.D.$_x$
		Lys	His	Arg	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe	
Casein	Mid jejunum	76.6[2]	82.8	76.2	69.5	70.6	75.9	81.4	70.7	-110.4	71.2	83.5	89.1	82.2	87.2	88.3	86.8	73.2± 4.3[3]
	Ileum	88.8	93.7	88.7	85.6	79.8	86.6	88.5	90.7	- 10.6	81.9	90.1	93.1	91.2	93.2	92.3	93.6	86.3± 4.6
	Caecum	89.7	93.1	87.0	83.1	86.0	91.1	93.4	95.1	69.1	76.0	89.8	89.6	90.0	91.9	90.9	87.4	87.6± 2.3
	Feces	92.5	95.4	90.7	88.1	90.1	93.7	95.5	95.9	79.9	85.1	92.9	92.6	92.6	93.8	93.1	93.3	91.6± 1.9
Autoclaved Casein	Mid jejunum	43.2	38.9	53.4	25.6	39.2	46.6	42.6	36.1	-153.4	37.3	55.9	58.2	50.0	60.1	65.1	64.6	44.0±18.4
	Ileum	72.2	82.6	86.0	55.7	72.2	71.5	76.1	77.7	24.6	76.8	83.5	77.2	77.5	87.5	92.4	91.9	78.3± 8.9
	Caecum	67.0	82.8	88.7	57.6	74.3	79.9	78.0	81.8	61.2	68.8	81.1	76.7	73.8	85.8	89.7	88.3	77.1± 1.5
	Feces	76.0	83.0	81.0	69.1	77.1	81.9	85.4	88.4	66.3	69.7	81.9	80.1	79.1	86.0	87.7	87.2	79.9± 2.9
Soybean Isolate	Mid jejunum	60.0	66.2	69.2	45.2	40.2	37.2	54.0	20.7	- 14.9	52.2	60.0	44.1	62.8	69.0	67.1	70.9	52.7± 1.5
	Ileum	75.8	78.9	85.6	57.1	51.8	51.4	73.8	55.2	40.3	73.6	77.3	61.2	76.4	82.2	82.0	80.7	70.3± 3.6
	Caecum	75.3	84.0	87.7	71.1	61.5	66.2	78.8	77.2	66.5	69.8	75.4	64.2	74.8	81.2	81.9	80.1	74.7± 3.3
	Feces	84.8	89.2	92.8	84.0	72.2	81.0	88.6	86.1	80.0	77.0	82.3	66.5	84.8	88.2	86.2	88.1	84.1± 1.3
Zein	Mid jejunum	-443.8	36.1	- 22.0	18.5	3.9	34.2	37.4	22.3	-538.2	41.4	21.4	32.3	29.7	52.9	36.9	49.1	26.1±19.8
	Ileum	- 99.6	51.3	48.5	62.7	52.6	67.3	65.0	51.4	- 33.6	63.8	60.5	56.9	59.4	71.5	63.2	67.2	60.8±17.3
	Caecum	- 85.7	80.5	78.9	79.5	79.2	85.9	85.5	84.6	56.5	84.1	78.2	75.5	79.7	87.4	84.9	84.5	82.0± 3.9
	Feces	-155.2	83.4	75.5	66.4	69.4	80.0	83.2	86.7	44.2	81.2	73.6	61.9	75.5	84.6	81.0	84.6	78.5± 1.5
RSM-1	Mid jejunum	41.9	40.9	47.4	31.1	32.2	31.9	44.4	8.0	- 41.7	34.5	38.4	64.5	43.6	46.4	43.7	48.0	29.6±22.1
	Ileum	64.2	77.5	79.1	66.8	63.0	66.9	81.0	56.6	59.7	72.1	69.1	80.1	75.7	76.0	66.4	74.0	70.4± 2.3
	Caecum	67.1	79.2	80.7	66.5	67.4	72.4	83.5	73.7	72.0	63.9	70.1	69.1	70.2	73.8	64.3	71.5	73.0± 2.0
	Feces	69.2	80.3	82.2	68.7	70.1	74.2	84.9	78.5	73.0	66.5	69.5	59.9	70.8	75.1	64.7	72.9	74.3± 2.7
RSM-5	Mid jejunum	44.4	61.1	66.3	46.9	48.0	52.7	62.9	37.3	13.0	57.8	57.5	77.1	57.8	59.0	61.8	59.6	52.1±12.7
	Ileum	72.5	84.5	88.3	73.9	71.5	76.0	85.6	49.8	67.3	79.8	77.7	86.9	77.6	79.9	81.2	79.7	77.2± 5.3
	Caecum	64.5	83.7	85.3	66.0	67.7	70.6	82.7	72.0	70.8	69.5	69.3	75.2	66.4	71.8	71.2	68.9	73.5± 1.8
	Feces	69.5	84.0	82.9	68.4	70.9	74.6	84.8	78.9	74.2	70.6	73.1	71.3	69.8	74.8	74.9	74.7	74.2± 1.7

[1]All apparent digestibility coefficients of amino acids are corrected to 95% recovery of amino acids (Knipfel et al., 1971).

[2]Each digestibility value represents the mean of 3 observations. Individual digestibility data in each region of the intestinal tract and the feces were from the same pigs.

[3]Mean ± S.D.$_x$ for 3 pigs.

Table 24. True digestibility (%) of individual amino acids of rapeseed meal and other protein sources at various sites along the intestinal tract of growing pigs[1].

Dietary Regimen	Sample	Amino Acids[2]															Protein Dig. (%)
		Lys	His	Arg	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe	
Casein	Mid jejunum	84.5[2]	91.4	98.9	84.0	87.0	89.4	86.9	120.2	64.5	95.4	93.9	94.1	88.7	94.4	95.5	93.7	94.6± 4.3[3]
	Ileum	90.0	98.8	97.2	96.0	93.9	96.0	92.8	124.6	82.5	95.3	96.4	96.2	96.2	97.9	96.6	96.4	97.0± 2.8
	Caecum	96.8	98.6	96.8	94.5	96.7	97.9	97.7	102.3	100.8	92.3	96.7	96.7	96.9	97.8	96.4	93.8	96.2± 5.7
	Feces	98.9	100.4	98.9	98.0	99.5	100.1	99.2	98.1	97.5	99.8	99.3	99.3	99.5	99.1	98.1	98.7	99.6± 1.9
Autoclaved Casein	Mid jejunum	51.6	48.1	77.9	40.8	56.2	60.5	48.1	86.1	20.8	62.3	65.8	63.4	56.3	67.6	72.4	71.6	65.0±18.1
	Ileum	76.6	89.1	97.1	65.3	85.1	82.5	79.8	105.2	114.4	89.4	89.0	79.8	81.8	91.9	96.4	96.2	88.9± 8.7
	Caecum	75.2	88.7	99.2	69.6	85.4	87.0	82.3	89.8	104.4	84.6	87.9	84.1	80.6	90.8	95.3	94.7	85.6± 1.3
	Feces	83.4	88.4	89.9	79.6	86.6	88.6	89.0	90.7	79.0	84.9	88.2	86.7	85.9	91.5	92.8	92.7	86.9± 2.0
Soybean Isolate	Mid jejunum	69.6	75.0	79.4	54.1	61.4	53.0	60.5	124.7	72.8	72.5	74.9	56.6	70.5	78.0	76.6	77.7	74.1± 1.7
	Ileum	80.8	85.1	90.3	62.8	68.3	61.1	78.2	112.4	85.5	83.8	85.3	66.8	81.7	87.4	87.1	85.0	81.1± 3.5
	Caecum	84.7	89.5	92.1	78.1	75.7	74.2	84.0	93.9	83.0	82.6	85.2	82.2	83.1	88.6	89.2	86.3	83.4±
	Feces	93.2	94.3	96.5	90.1	84.6	86.9	92.9	90.8	89.2	89.3	91.4	83.0	93.1	94.4	92.8	93.0	91.2± 1.5
Zein	Mid jejunum	-226.4	54.8	33.2	37.7	29.5	48.5	39.1	82.1	-257.2	49.7	40.3	44.6	38.1	56.3	44.9	54.2	47.3±19.2
	Ileum	54.4	64.5	73.5	74.8	72.0	76.2	66.1	84.3	111.2	68.0	70.8	63.4	65.2	73.5	67.7	70.4	71.5±17.0
	Caecum	98.4	93.3	100.9	94.0	94.9	93.3	91.8	97.2	112.6	91.4	92.2	89.1	91.0	91.8	92.1	91.3	92.6± 3.8
	Feces	56.9	86.6	86.6	83.0	84.3	87.0	86.8	89.5	81.1	86.6	85.1	80.0	84.9	87.3	86.7	88.8	85.8± 1.0
RSM-1	Mid jejunum	50.8	50.0	61.7	45.3	47.9	48.7	51.0	87.4	13.9	52.0	51.4	73.6	51.7	56.1	57.6	57.3	51.0±21.9
	Ileum	69.5	84.0	85.6	75.8	74.9	77.3	85.4	99.3	92.5	80.9	77.1	84.7	81.2	81.6	74.0	79.8	80.4± 2.3
	Caecum	77.0	85.0	86.8	77.6	79.1	80.9	88.7	86.3	84.0	75.0	78.7	62.2	78.9	81.7	75.0	80.1	81.7± 2.1
	Feces	78.2	85.6	87.4	78.4	79.1	82.1	89.2	82.0	79.6	77.2	77.4	72.2	79.6	81.8	74.3	80.2	81.4± 2.8
RSM-5	Mid jejunum	55.0	69.6	79.4	60.8	63.3	65.9	69.7	100.7	76.4	73.8	70.3	79.5	66.4	68.6	73.0	68.5	77.3±12.9
	Ileum	78.0	90.6	94.3	82.7	83.1	86.3	90.2	93.1	100.0	87.9	84.5	90.3	83.4	85.5	87.4	85.3	88.0± 4.4
	Caecum	74.9	89.1	90.9	76.9	77.7	79.3	88.1	84.6	82.8	79.6	77.7	85.0	75.7	79.7	79.8	76.7	82.0± 1.9
	Feces	79.0	88.9	87.7	81.3	79.6	82.9	89.3	82.4	80.8	80.3	80.9	80.6	79.0	81.0	82.7	81.7	81.3± 1.8

[1]All true digestibility coefficients of amino acids are corrected to 95% recovery of amino acids.

[2]Each value represents the mean of 3 observations. Digestibility data in each region of the intestinal tract and the feces were from the same pigs.

[3]Mean ± $S.D._{\bar{x}}$ for 3 pigs.

caecum compared to that from the ileum for pigs fed the protein-free diet (Table 20) coincides with the fact that absorption of bile salts takes place in the ileum. The effect of endogenous glycine on the apparent absorption of glycine was most pronounced with casein, autoclaved casein and zein where the level of glycine was relatively low in the diet. The apparent digestibility of proline also tended to improve from the ileum to the caecum (Table 23). As with glycine there was an appreciable decrease in the proline content of digesta from the ileum to the caecum of pigs fed the protein-free diet (Table 20). The marked effect of endogenous proline and glycine on the apparent availability of these amino acids is demonstrated by the fact that the true digestibility of these amino acids (Table 24) often exceeded 100% with digesta from the ileum and caecum.

Of particular interest is the fact that true digestibility coefficients improved from the ileum to the feces for all of the amino acids in zein whereas with soybean protein and autoclaved casein improvement in digestibility occurred only with certain amino acids (Table 24). Improvement in the digestibility coefficients was particularly striking for amino acids which were relatively poorly absorbed in the small intestine whereas little change occurred between the ileum and feces for amino acids that were well digested. These observations suggest that fermentation may be more extensive for amino acids which are poorly digested in the small intestine. This might lead to an overestimation of digestibility for amino acids which are slowly digested. Similarly Cho and Bayley (1972) indicated that digestibilities based on fecal analysis would overestimate the true availability of several amino acids in soybean meal.

In addition, the fact that less fermentation seemed to occur with rapeseed meal than with soybean protein and zein (Table 24) could be due to the nature of the residual nitrogenous and fibrous material in the large intestine of pigs fed rapeseed meal. Recovery of nitrogen as ninhydrin-positive material with the diet and digesta from ileum, caecum and feces of pigs fed RSM was significantly ($P<0.05$) lower than that found with the other proteins (Table 20). These results suggest that protein from rapeseed meal and digesta from pigs fed this protein contain a greater proportion of non-protein nitrogen. A similar observation also was reported by Cho and Bayley (1970, 1972) and Tao et al. (1971). Since amino acid analyses in the present experiment were corrected to 95% recovery of nitrogen as ninhydrin-positive material the digestibility of amino acids with rapeseed meal have been somewhat underestimated relatively to those in the other proteins. Furthermore, as indicated in Table 17, dry matter digestibility of rapeseed meal was significantly lower ($P<0.05$) than that of the other proteins in the ileum, caecum and feces whereas the coefficients of apparent protein digestibility for rapeseed meals did not differ from that of soybean isolate and zein in the ileum but was significantly lower than that of soybean isolate in the feces. Michel (1965) postulated that the catabolic activity of the microflora in the caecum and colon varies not only with the levels of small peptides and free amino acids but also with the presence of carbohydrates. In the absence of carbohydrates bacteria will use amino acids for energy purposes. Thus it is conceivable that less fermentation of protein might occur with rapeseed meal since there was a greater proportion of fibrous or carbohydrate material in the lower part of the intestinal

tract of pigs fed this diet (Table 17). Besides, the presence of fiber may prevent or at least restrict rapid fermentation of the protein.

Digestibility values for the individual amino acids in the various proteins followed a similar pattern in the mid jejunum and ileum although there was, with the exception of casein, a marked improvement in digestibility values from the mid jejunum to the ileum. Almost complete absorption had occurred for all amino acids from casein by the time digesta reached the mid jejunum. Thus high amino acid digestibility coefficients in the ileum of pigs fed casein are undoubtedly related to the relatively high digestibility of amino acids from this protein in the mid jejunum. Except for proline and glycine true digestibility coefficients for the individual amino acids were consistently higher in the mid jejunum of pigs fed casein than for those fed the other protein sources.

However with proteins other than casein, there were some rather marked exceptions to the similarity of amino acid digestibilities in the mid jejunum relative to those in the lower regions of the intestinal tract. True digestibility values for lysine, histidine and glutamic acid were lower than the true digestibility of protein in the mid jejunum of pigs fed autoclaved casein but were similar to the digestibility of protein in the feces. Similarly true digestibility coefficients of serine, aspartic acid and glutamic acid were lower than that of the protein in the mid jejunum of pigs fed soybean protein but did not differ from that of protein in the feces. With rapeseed meal there was a poor relationship between the high availability of methionine in the mid jejunum and the lower relative digestibility of this amino

acid in the feces. Similarly the high digestibility of glutamic acid in the feces of pigs fed rapeseed meal was not preceded by a high digestibility coefficient for this amino acid in the mid jejunum. Furthermore, apparent and true digestibility coefficients of proline and glycine in the mid jejunum of pigs fed the various protein sources bore little relationship to the values obtained in the feces.

In summary, there was generally good agreement between the relative digestibility values of amino acids in the ileum and that observed in the caecum and feces, except in the case of proline, glycine, methionine, aspartic acid and glutamic acid. Thus amino acid digestibilities based on fecal analysis will probably give a reasonably satisfactory estimation of the true availability of most amino acids. The present results do not support the suggestion (Cho and Bayley, 1972; Salter and Coates, 1971; Payne et al., 1968) that extensive fermentation of protein occurs in the large intestine. Of the proteins studied in this experiment only soybean protein and zein showed marked improvement in apparent and true digestibility between the ileum and feces. The gradual improvement in apparent and true digestibility of total protein and of individual amino acids from the ileum to the feces of pigs fed soybean protein together with a marked improvement in the true digestibilities of aspartic acid and serine suggest that fermentation may have a considerable effect on amino acid availability in soybean protein when based on fecal analyses. In addition the appreciable changes in the digestibilities of proline, glycine and methionine between the ileum and the feces for most protein sources suggests that caution should be exercised in the interpretation of digestibility results for these amino acids based on fecal analyses.

4.2.5 Effect of dietary proteins and amino acid supplementation upon plasma amino acid pattern.

In general there was no evidence of a close relationship between the plasma amino acid levels of pigs and the amino acid composition of the dietary proteins fed these pigs although in some cases there were obvious effects of dietary amino acids and amino acid supplementation on the plasma amino acid (PAA) pattern, particularly postprandial amino acid values (Table 25).

Lysine and methionine supplementation resulted in higher fasting plasma levels of these amino acids as well as higher fasting levels of several other amino acids with most of the protein sources. Plasma methionine levels for pigs fed casein, and zein supplemented with 0.2 and o.3% methionine, respectively, were more than twice those of pigs fed the non-supplemented diets. Similarly, supplementation of autoclaved casein with lysine and methionine and rapeseed meal with lysine resulted in higher fasting levels of these amino acids in the plasma of pigs fed these proteins. The relatively high level of lysine and proline in the plasma of pigs fed casein compared to that of pigs fed the other protein sources coincided with the relatively high content of these amino acids in this protein source. The high content of arginine in soybean protein also was accompanied by a higher level of arginine in the plasma of pigs fed this protein. Similarly the relatively low level of lysine and methionine in zein and methionine in soybean protein resulted in lower levels of these amino acids in the plasma. On the other hand, the relatively high level of alanine and leucine in zein compared to the other protein sources was not accompanied by higher levels of these amino acids in pigs fed the zein diet.

Table 25. Fasting and postprandial plasma amino acid levels (umoles/100 ml) of pigs fed different protein diets with or without amino acid supplementation.

Dietary Regimen	Amino Acid Suppl.[1]	Time of Collection[2]	Amino Acids (umoles/100 ml of plasma)															
			Lys	His	Arg	Asp& Thr	Ser	Pro	Glu	Gly	Ala	Val	Cys	Met	Ile	Leu	Tyr	Phe
Casein		1-BM	17.5[3]	8.4	9.8	30.9	10.5	34.5	24.7	51.1	23.6	10.3	4.3	3.3	10.9	14.7	8.0	4.8
	-	1-AM	19.7	8.3	11.8	43.4	15.6	49.2	37.6	62.4	33.9	26.5	4.6	4.1	16.1	21.4	11.7	8.5
		3-AM	20.4	8.8	11.6	69.4	15.9	49.5	33.1	58.9	35.8	35.3	4.4	3.4	13.9	20.4	11.3	8.0
	0.2% DL-Met	1-BM	18.5	12.3	10.7	35.1	15.9	42.1	50.9	62.8	43.1	34.7	7.5	7.6	15.4	20.0	18.7	9.6
		1-AM	16.9	8.8	9.0	35.2	11.8	47.1	58.1	55.8	42.2	32.6	3.5	5.8	13.0	19.5	14.6	6.8
		3-AM	17.1	7.3	7.3	40.3	13.5	32.4	33.9	39.7	52.8	38.9	3.6	6.4	13.4	20.5	14.2	7.6
Autoclaved Casein		1-BM	12.7	6.4	11.6	20.7	9.8	31.9	36.1	56.4	20.6	24.9	1.5	3.9	7.9	14.4	13.9	7.5
	-	1-AM	10.1	4.5	9.3	18.7	9.5	26.1	30.6	49.3	20.9	19.8	3.7	4.0	6.2	11.2	8.9	5.5
		3-AM	7.8	4.2	7.7	18.7	8.2	23.3	32.3	42.7	20.7	20.0	3.8	4.0	7.0	10.6	8.4	6.4
	0.2% L-Lys	1-BM	16.9	8.0	9.2	24.5	10.0	25.2	24.7	55.3	29.3	26.5	3.3	5.8	8.1	13.7	10.9	5.1
	0.3% DL-Met	1-AM	22.5	10.0	11.2	15.5	8.5	30.6	34.5	48.7	35.9	28.6	6.6	8.4	9.1	18.2	11.4	7.4
		3-AM	17.7	8.3	12.4	13.7	8.7	29.8	27.6	40.4	31.2	24.6	6.8	8.1	9.5	16.5	11.0	7.4
Soybean Protein		1-BM	8.7	7.0	12.1	23.0	10.8	16.7	33.6	50.8	27.1	14.9	2.1	2.5	7.4	13.0	5.5	6.0
	-	1-AM	11.2	7.7	20.4	23.7	12.2	18.0	27.4	44.6	28.7	22.1	0.9	2.1	8.4	14.9	7.0	8.4
		3-AM	10.4	5.5	15.4	22.4	11.3	16.3	23.5	26.1	25.0	20.9	0	0.7	8.2	11.3	5.4	6.4
	0.3% DL-Met	1-BM	6.1	6.8	13.8	13.9	8.2	18.9	30.1	44.6	25.7	25.8	3.4	3.1	11.4	14.9	6.6	7.1
		1-AM	11.0	7.3	21.4	19.3	13.6	34.4	43.1	56.2	45.3	38.9	5.3	5.6	17.5	24.6	9.8	13.0
		3-AM	7.6	8.3	16.8	15.9	10.4	27.7	28.2	45.5	36.6	29.4	1.0	4.6	14.0	18.5	8.2	10.1
Zein		1-BM	6.2	6.1	7.0	23.1	10.8	12.8	17.6	42.7	20.9	11.9	3.2	1.7	4.9	12.4	5.5	6.1
	-	1-AM	4.3	5.9	6.7	23.6	13.8	25.0	21.9	36.7	29.9	9.6	0	2.6	5.0	21.3	6.4	7.4
		3-AM	3.0	7.4	7.4	18.3	12.7	14.5	17.9	39.7	28.7	11.7	1.5	1.2	5.0	22.5	8.3	8.5
	0.6% L-Lys	1-BM	11.3	5.7	10.7	21.7	9.7	15.8	23.7	41.1	24.3	13.3	4.0	4.1	5.9	12.7	5.5	5.3
	0.3% DL-Met	1-AM	13.7	2.8	9.6	20.7	13.5	26.5	35.0	36.3	34.6	11.9	2.4	5.1	7.1	27.5	10.4	11.4
	0.06% L-Tryp	3-AM	13.4	5.4	7.7	18.3	11.8	35.3	45.0	31.7	48.7	9.5	1.1	5.2	6.4	34.7	11.1	10.6
Rapeseed meal-1		1-BM	9.9	6.7	8.2	17.4	11.7	16.1	26.7	58.8	30.3	19.5	1.3	2.9	5.1	10.0	2.9	4.3
	-	1-AM	12.7	7.0	14.1	25.1	13.7	20.8	31.2	53.1	26.2	22.5	2.2	3.7	8.8	12.6	4.3	6.5
		3-AM	9.7	7.0	12.4	19.7	10.1	19.2	22.0	42.5	28.0	19.0	1.3	2.2	7.1	9.2	3.6	5.2
	0.25% L-Lys	1-BM	15.3	8.4	14.1	24.1	12.6	25.8	39.1	78.5	41.3	31.3	3.9	3.8	11.2	16.1	3.1	5.6
		1-AM	18.2	8.7	17.3	31.1	18.0	20.5	54.6	97.4	70.2	43.6	6.2	4.7	14.4	22.1	3.8	8.9
		3-AM	14.2	7.4	11.9	22.0	11.1	23.5	35.0	55.0	38.1	28.2	0.7	3.7	9.0	13.1	6.4	5.2
Protein-Free		1-BM	24.8	8.9	8.6	15.8	17.9	14.3	17.3	77.6	35.5	16.0	4.9	2.9	8.4	12.3	3.4	5.2
		1-AM	17.6	6.3	7.0	15.6	14.2	23.0	20.4	61.5	40.4	13.2	3.0	3.1	5.8	7.9	3.0	3.6
		3-AM	12.5	6.4	2.3	10.1	8.7	19.3	12.8	48.1	32.6	9.7	2.0	2.4	4.4	6.2	1.8	3.3

[1]Amino acid supplementation is expressed as % of dry matter of the diets.

[2]The time of collection of blood samples are expressed by the following abbreviations:

1-BM = Blood collected at fasting one hour before the meal.
1-AM = One hour postprandial.
3-AM = Three hours postprandial.

[3]All plasma amino acid values are means for 2 pigs.

There were no consistent changes in PAA patterns in response to feeding the various protein sources. Postprandial PAA levels of pigs fed the methionine supplemented casein diet decreased whereas PAA levels increased for pigs fed the non-supplemented casein diet. By contrast, there was a general increase in PAA levels of pigs fed soybean protein supplemented with methionine especially at one hour postprandial, whereas there was a decrease in the plasma levels of many amino acids for pigs fed the non-supplemented soybean protein diet.

Postprandial changes in PAA levels in response to the various protein sources was highly variable and did not appear to depend on the amino acid content of the dietary proteins (Table 26). Nevertheless, amino acid supplementation of the dietary proteins with known limiting amino acids generally prevented the postprandial decrease in PAA levels that accompanied feeding of the non-supplemented diets. In fact lysine supplementation of autoclaved casein and zein and methione supplementation of soybean increased the postprandial plasma level of these amino acids for pigs fed these diets. Similarly, the postprandial increase in plasma alanine, leucine and phenylalanine of pigs fed zein and arginine in pigs fed soybean protein coincided with the relatively high levels of amino acids in the respective diets.

In general the limiting amino acid(s) in the different proteins was indicated by a decrease in the postprandial concentration of these amino acids in pigs fed the non-supplemented diet. The plasma level of methionine for pigs fed soybean protein decreased from a fasting level of 2.5 umoles/100 ml to postprandial values of 2.1 and 0.7 umoles/100 ml at 1 and 3 hours, respectively, after feeding. Similarly plasma lysine content of pigs fed zein decreased from a fasting value of 6.2 to levels

Table 26. Postprandial changes in plasma amino acids (umoles/100 ml) relative to fasting values of pigs fed different protein diets with or without amino acid supplementation.

Dietary Regimen	Amino Acid Supp.[1]	Time of Collection[2]	Amino Acids (umoles/100 ml. of plasma)														
			Lys	His	Arg	Asp & Thr	Ser	Pro	Glu	Gly	Ala	Val	Cys	Met	Ile	Leu	Tyr	Phe
Casein	-	1-AM	2.2[3]	- 0.1	2.0	12.5	5.1	14.7	12.9	11.3	10.3	16.2	0.5	0.8	5.2	6.7	3.7	3.7
		3-AM	2.9	0.4	1.8	38.5	5.4	15.0	8.4	7.8	12.2	25.0	0.3	0.1	3.0	5.7	3.3	3.2
	0.2% DL-Met	1-AM	- 1.6	- 3.5	- 1.7	0.1	- 4.1	5.0	7.2	- 7.0	- 0.9	- 2.1	- 4.0	- 1.8	- 2.4	- 0.5	- 4.1	- 2.8
		3-AM	- 1.4	- 5.0	- 3.4	5.2	- 2.4	- 9.7	-17.0	-23.1	9.7	4.2	- 3.9	- 1.2	- 2.0	0.5	- 4.5	- 2.0
Autoclaved Casein	-	1-AM	- 2.6	- 1.9	- 2.3	- 2.0	- 0.3	- 5.8	- 5.5	- 7.1	0.3	- 5.1	2.2	0.1	- 1.7	- 3.2	- 5.0	- 2.0
		3-AM	- 4.9	- 2.2	- 3.9	- 2.0	- 1.6	- 8.6	- 3.8	-13.7	0.1	- 4.9	2.3	0.1	- 0.9	- 3.8	- 5.5	- 1.1
	0.2% L-Lys 0.3% DL-Met	1-AM	5.6	2.0	2.0	- 9.0	- 1.5	5.4	9.8	- 6.6	6.6	2.1	3.3	2.6	1.0	4.5	0.5	2.3
		3-AM	0.8	0.3	3.2	-10.8	- 1.3	4.6	2.9	-14.9	1.9	- 1.9	3.5	2.3	1.4	2.8	0.1	2.3
Soybean Protein	-	1-AM	2.5	0.7	8.3	0.7	1.4	1.3	- 6.2	- 6.2	1.6	7.2	- 1.2	- 0.4	1.0	1.9	1.5	2.4
		3-AM	1.7	- 1.5	3.3	- 0.6	0.5	- 0.4	-10.1	-24.7	- 2.1	6.0	- 2.1	- 1.8	0.8	- 1.7	- 0.1	0.4
	0.3% DL-Met	1-AM	4.9	0.5	7.6	5.4	5.4	15.5	13.0	11.6	19.6	13.1	1.9	2.5	6.1	9.7	3.2	5.9
		3-AM	1.5	1.5	3.0	2.0	2.2	8.8	- 1.9	0.9	10.9	3.6	- 2.4	1.5	2.6	3.6	1.6	3.0
Zein	-	1-AM	- 1.9	- 0.2	- 0.3	0.5	3.0	12.2	4.3	- 6.0	9.0	- 2.3	- 3.2	0.9	0.1	8.9	0.9	1.3
		3-AM	- 3.2	1.3	0.4	- 4.8	1.9	1.7	0.3	- 3.0	7.8	- 0.2	- 1.7	- 0.5	0.1	10.1	2.8	2.4
	0.6% L-Lys 0.3% DL-Met 0.06% L-Tryp	1-AM	2.4	- 2.9	- 1.1	- 1.0	3.8	10.7	11.3	- 4.8	10.3	- 1.4	- 1.6	1.0	1.2	14.8	4.9	6.1
		3-AM	2.1	- 0.3	- 3.0	- 3.4	2.1	19.5	21.3	- 9.4	24.4	- 3.8	- 2.9	1.1	0.5	22.0	5.6	5.3
RSM-1	-	1-AM	2.8	0.3	5.9	7.7	2.0	4.7	4.5	- 5.7	- 4.1	3.0	0.9	0.8	3.7	2.6	1.4	2.2
		3-AM	- 0.2	0.3	4.2	2.3	- 1.6	3.1	- 4.7	-16.3	- 2.3	- 0.5	0.0	- 0.7	2.0	- 0.8	0.7	0.9
	0.25% L-Lys	1-AM	2.9	0.3	3.2	7.0	5.4	- 5.3	15.5	18.9	28.9	12.3	2.3	0.9	3.2	6.0	0.7	3.3
		3-AM	- 1.1	- 1.0	- 2.2	- 2.1	- 1.5	- 2.3	- 4.1	-23.5	- 3.2	- 3.1	- 3.2	- 0.1	- 2.2	- 3.0	3.3	- 0.4
Protein-Free	-	1-AM	- 7.2	- 2.6	- 1.6	- 0.2	- 3.7	8.7	3.1	-16.1	4.9	- 2.8	- 1.9	0.2	- 2.6	- 4.4	- 0.4	- 1.6
		3-AM	-12.3	- 2.5	- 6.3	- 5.7	- 9.2	5.0	- 4.5	-29.5	- 2.9	- 6.3	- 2.9	- 0.5	- 4.0	- 6.1	- 1.6	- 1.9

[1]Amino acid supplementation is expressed as % of dry matter in the diet.

[2]The following abbreviations are for: 1-AM = One hour postprandial; 3-AM = Three hours postprandial.

[3]All values are means for 2 pigs.

of 4.3 and 3.0 umoles/100 ml at 1 and 3 hours postprandial. Lysine appeared to be somewhat limiting in autoclaved casein as indicated by the gradual decrease in postprandial plasma lysine levels. On the other hand, no amino acids in rapeseed meal appear to be limiting for the pig.

The present results also suggest that the digestion of the proteins fed in this experiment was very rapid since the postprandial PAA levels appear to reach a maximum within 1 hour of feeding. A similar observation was also reported by Boomgaardt (1969). The gradual decrease in PAA levels for pigs fed the protein-free diet probably resulted from the removal of amino acids necessary for the secretion of endogenous protein. The relatively high fasting plasma lysine value for pigs fed the protein-free diet was of particular interest. Charkey et al. (1953, 1954), Gray et al. (1960), Hill and Olsen (1962) also reported that in chicks the removal of feed resulted in a marked elevation in the concentration of lysine. A possible explanation to this phenomenon is the fact that lysine is particularly resistant to deamination and thus accumulates in the blood during tissue breakdown.

Nevertheless, plasma amino acid pattern appears to give useful information concerning whether some amino acids are limiting in the diet as the result of their poor concentration in the diet. The present study suggests that the plasma concentration of individual amino acids depends to a certain degree on the amount present in the diet. However, in view of the many factors which influence PAA patterns such as rate of absorption from the small intestine, rate of removal by the tissues, catabolism in the liver, etc., it is difficult to relate the PAA to the general availability of the individual amino acids.

4.2.6 Effect of amino acid supplementation on utilization of various proteins.

Amino acid supplementation of the various proteins had no significant effect on feed intake although lowest intake occurred with the diet containing autoclaved casein and highest with RSM-4 (Bronowski), a low-thioglucoside B. napus variety. Amino acid supplement improved significantly ($P < 0.05$) weight gains for piglets fed soybean isolate and zein. On the other hand, the lysine supplementation (0.25%) in the rapeseed meal diets had no apparent effect on the weight gain of piglets fed RSMs. Although addition of amino acids did not influence apparent protein digestibility, amino acid supplementation markedly improved nitrogen retention for several of the proteins. Nitrogen retention was increased slightly by the addition of 0.2% methionine to casein. With soybean protein, 0.3% DL-methionine doubled nitrogen retention whether expressed as total N retained or as % of N intake or % of N absorbed. Similarly nitrogen retention with zein diet was substantially improved ($P < 0.05$) by the addition of lysine, methionine and tryptophan. By contrast the addition of 0.25% lysine to RSM diets or 0.2% lysine and 0.3% methionine in the autoclaved casein diet had no apparent effect on nitrogen retention.

In conclusion, amino acid supplementation had a beneficial effect on the utilization of protein from casein, soybean protein and zein. This observation is in agreement with the fact that the methionine and lysine content of these proteins is insufficient to meet the amino acid requirement of piglets when fed diets containing approximately 16% protein. However, contrary to the results of Kratzer (1954), lysine supplementation did not appear beneficial for the overall

Table 27. Effect of protein source and amino acid supplementation upon feed intake, weight gain, apparent protein digestibility and nitrogen retention of growing pigs.

Protein Source	Amino Acid Suppl.[1]	Feed Intake (gm/pig/5 days)	Weight Gain (Kg/pig/5 days)	Apparent Protein Digestibility %	Nitrogen Retention		
					gm/pig/5 days	% N intake	% N absorbed
Casein	(-)	2088[2]±1000abcd[3]	0.48±0.11cde	90.9±1.5a	28.2±12.9abc	54.2± 1.3ab	59.6± 2.5ab
	(+)	1756 ± 550bcd	0.80±0.16bcde	92.4±1.0a	31.4± 9.1ab	66.3± 0.5a	71.3± 0.9a
Autoclaved Casein	(-)	1422 ± 242d	0.30±0.29de	79.1±1.9bcd	18.2± 6.6cd	50.0±10.0ab	63.2±11.2ab
	(+)	1616 ±1195bcd	0.45±0.13cde	79.6±3.9bcd	17.0±13.0cd	40.2± 1.6bc	50.5± 1.0bc
Soybean Isolate	(-)	1942 ± 108abcd	0.44±0.21de	85.0±0.4b	10.2± 4.3de	20.9± 7.9d	24.6± 9.2e
	(+)	2618 ± 614abc	1.02±0.31abc	83.4±0.1bc	25.6± 0.6abc	39.9± 9.7bc	47.9±11.7bcd
Zein	(-)	1537 ± 392cd	-0.28±0.01d	77.8±0.2bc	- 0.8± 4.1e	- 0.9±10.4e	- 1.1±13.3f
	(+)	1738 ± 249bcd	0.27±0.03e	83.9±5.7cd	11.5± 2.2de	25.2± 1.3cd	30.1± 3.5de
RSM-1	(-)	2519 ±1071abc	1.41±0.46a	75.8±0.5d	24.8±17.2abc	36.9±11.5bcd	48.7±15.5bcd
	(+)	1620 ±1207bcd	0.86±0.79bcd	74.7±4.9d	10.5± 5.4de	28.7± 8.3cd	37.9± 8.9cde
RSM-4	(-)	2663 ± 603ab	1.02±0.16abc	78.2±2.7cd	32.9± 4.4a	48.7± 4.6b	62.1± 3.8ab
	(+)	2885 ±1004a	1.11±0.48ab	76.8±1.8d	35.0±11.4a	47.2± 1.5b	61.5± 3.2ab
RSM-5	(-)	2171 ± 546abcd	1.20±0.23ab	75.1±0.1d	23.2± 7.5abcd	42.2± 7.4bc	56.2± 4.1abc
	(+)	1839±1192abcd	0.68±0.32bcde	74.4±2.6d	19.5±13.3bcd	40.7± 1.9bc	54.8± 4.4abc

[1]See Table 6 for details on amino acid supplementation; (-) = no amino acid supplementation, (+) = with amino acid supplementation.

[2]Mean ± $S.D._{\bar{x}}$ for 2 pigs.

[3]Any two means not followed by the same letters are significantly ($P < 0.05$) different.

utilization of RSM. The lysine content of this protein appears to be sufficient to meet the lysine requirement of the piglet. Thus the lysine availability does not appear to be a limiting factor in the utilization of RSM. Furthermore our results do not agree with those reported by Tao et al. (1971) concerning the fact that lysine supplementation increases the protein digestibility of rapeseed meal.

4.2.7 Trypsin and chymotrypsin activity

Trypsin and chymotrypsin activity was determined in the soluble fraction from the mid jejunum in an attempt to establish if there was any relationship between the rate of absorption of the various proteins and proteolytic activity in the intestinal lumen. Trypsin activity per 100 mg of dry matter was appreciably higher for pigs fed autoclaved casein, soybean protein and zein than for those fed rapeseed meal, casein or a protein-free diet (Table 28). These results do not support those of Snook and Meyer (1964a, 1964b) and Snook (1965) that the ingestion of casein resulted in greater trypsin activity than ingestion of zein or a protein-free diet. Low trypsin activity in the digesta of pigs fed the casein diet may reflect autocatalytic breakdown of trypsin since a substantial portion of the casein had been digested and absorbed by the time digesta reach the mid jejunum. However, the same argument would not apply to the group fed rapeseed meal although stability of the enzyme may be related more to the soluble protein content of the digesta than total protein content. In addition, the relatively high level of dry matter in the mid jejunum of pigs fed rapeseed meal also would contribute to the lower proteolytic enzyme concentration found in samples of dried digesta from these pigs. There was no indication that the lower digestibility of rapeseed meal was due to the presence of a trypsin inhibitor in this protein.

Table 28. Trypsin and chymotrypsin activity in digesta from the mid jejunum of pigs fed rapeseed meals and other protein sources.

DIETARY REGIMEN	ENZYME ACTIVITY (Units/100 mg of D.M.)	
	TRYPSIN[1]	CHYMOTRYPSIN[2]
Casein	222± 71[3]	26±20
Autoclaved casein	318± 44	34±10
Soybean isolate	436±165	39±17
Zein	313±111	21± 2
RSM-1	175± 79	26± 8
RSM-4	170± 62	22± 6
RSM-5	112± 82	14± 9
Protein-free	174± 63	7± 2

[1]Activity for trypsin is expressed in micromoles TAME hydrolyzed/min at 30°C.

[2]Activity for chymotrypsin is expressed in micromoles BTEE hydrolyzed/min at 30°C.

[3]Mean ± $S.D._{\bar{x}}$ for 2 pigs.

The chymotrypsin activity for pigs fed the protein-containing diets was about 1/10 that of trypsin but unlike trypsin it was higher for all groups fed protein than for the group fed the protein-free diet. Chymotrypsin activity for pigs fed rapeseed meal was similar to that of pigs fed casein and zein but slightly lower than that found with autoclaved casein and soybean. The lower relative activity of chymotrypsin as compared to that reported in other studies (Snook and Meyer, 1964a, 1964b) might be due to greater inactivation of this enzyme as the result of lyophylization of the digesta samples.

4.3 Experiment III. Study of the effect of hull-removal on the utilization of rapeseed protein

4.3.1 Protein and dry matter digestibility

Apparent and true protein digestibility of all rapeseed samples except the low-hull fraction of Bronowski was significantly ($P<0.05$) lower than that of either soybean meal or soybean isolate (Table 29). There was no difference in protein digestibility between soybean meal and soybean isolate. However, removal of the hull fraction in RSM resulted in an overall improvement ($P<0.05$) in protein digestibility although apparent protein digestibility was significantly ($P<0.05$) higher only with the B. napus and B. campestris meals. These results suggest that the fiber-rich hull fraction in RSM either interferes with protein digestion or that the protein associated with the hull fraction is more resistant to digestion than the protein in the accepts fraction. Dry matter digestibility of the regular RSM followed a similar pattern to protein digestibility whereas

Table 29. Apparent and true protein digestibilities (%) of regular and low-fiber rapeseed meals, soybean meal and soybean isolate and dry matter digestibility of these diets by the growing pig[1].

	Protein Source											
				Rapeseed								
	Soybean			B. campestris			B. napus			B. napus cv. Bronowski		
	Meal		Isolate	Meal		Low Fiber	Meal		Low Fiber	Meal		Low Fiber
Digestibility of Protein (%)												
Apparent	83.4[2] ± 1.8	(83.5)[3]	83.5 ± 1.7	73.2 ± 2.4	(76.4)	79.6 ± 2.3	75.9 ± 2.1	(77.5)	79.1 ± 2.2	77.9 ± 3.9	(79.4)	80.9 ± 1.5
	a[4]	A[5]	a	d	C	b	cd	BC	b	bc	B	ab
True[6]	90.2 ± 1.8	(90.5)	90.7 ± 1.7	80.0 ± 2.4	(83.2)	86.3 ± 2.3	83.2 ± 2.1	(84.6)	85.9 ± 2.2	84.3 ± 3.9	(85.9)	87.5 ± 1.5
	ab	A	a	e	C	cd	de	BC	cd	d	B	bc
Digestibility of Dry Matter (%)	86.0 ± 2.6	(85.2)	84.3 ± 2.1	75.3 ± 1.9	(77.7)	80.0 ± 1.8	78.4 ± 3.4	(79.8)	81.1 ± 0.4	80.5 ± 1.7	(80.4)	80.2 ± 2.4
	a	A	a	c	C	b	b	B	b	b	B	b

[1]Five-day fecal collection.

[2]Mean ± $S.D._{x}$ for 5 pigs.

[3]Mean value for the two adjacent groups.

[4]Any mean not followed by the same small letter is significantly ($P < 0.05$) different.

[5]Any mean not followed by the same capital letter is significantly ($P < 0.05$) different.

[6]Metabolic fecal nitrogen was based on the nitrogen/Cr_2O_3 ratio for 3 pigs fed a protein-free diet in Experiment II.

there was a poor relationship between the digestibility of dry matter and protein with the low-fiber RSM. The correlation coefficients between dry matter and apparent protein digestibility were 0.68 ($P<0.01$) and 0.36 for the regular rapeseed meals and the low-hull accepts fractions, respectively. This close relationship between dry matter and protein digestibility with the regular RSM is another indication that the non-protein material, and probably the fiber, present in rapeseed meal interferes somewhat with protein digestion.

Although protein digestibility on the basis of a five-day fecal collection was significantly ($P<0.05$) higher for soybean protein than for rapeseed protein, no appreciable differences were found between these two proteins for digestibilities calculated on the basis of digesta obtained from the small intestine (Tables 30, 31, 32). Similarly, removal of the hull in rapeseed meal had no apparent effect on protein digestibility coefficients in the small intestine. Thus the present results correspond to those obtained in the second experiment where no differences were found in the rate of digestion of soybean and rapeseed protein in the small intestine.

There was an appreciable increase in the digestibility of soybean protein from the ileum to the caecum in contrast to only a slight increase with the rapeseed meal diets (Tables 30 and 31). There was also an additional increase in protein digestibility for all dietary treatments as digesta passed through the large intestine. These observations agree with those of the previous experiment (Experiment II) although in Experiment II the increase in protein digestibility for soybean was more gradual than in the present experiment. Of particular interest was the fact that the improvement in protein digestibility

Table 30. Apparent protein digestibility (%) of regular and low-fiber rapeseed meals, soybean meal and soybean isolate at various sites along the alimentary tract of pigs.

	Protein Source							
			Rapeseed					
	Soybean		B. campestris		B. napus		B. napus cv. Bronowski	
Source of Digesta	Meal	Isolate	Meal	Low Fiber	Meal	Low Fiber	Meal	Low Fiber
Stomach	12.5[1] ±11.6 a[2]	3.2 ±11.5 a	15.7 ± 7.2 a	11.3 ±11.0 a	13.7 ±12.6 a	20.4 ±15.4 a	12.4 ±18.0 a	16.7 ±21.9 a
Mid jejunum	52.3 ± 5.8 a	51.8 ± 8.3 a	53.8 ± 7.0 a	55.5 ± 5.6 a	55.1 ± 5.0 a	41.3 ±13.0 a	45.2 ±11.1 a	51.2 ± 6.5 a
Ileum	70.8** ± 4.7 a	65.8** ± 6.8 a	69.8 ± 2.4 a	72.5[3] ± 3.2 a	69.9[3] ± 4.5 a	63.0[3]* ±15.6 a	71.3 ± 5.5 a	66.1* ± 8.3 a
Caecum	80.2 ± 2.3 a	81.8 ± 2.5 a	72.0 ± 1.7 bc	75.5 ± 2.6 b	72.7 ± 2.9 bc	72.3 ± 6.0 bc	72.0 ± 2.3 bc	70.3 ± 4.1 c
Rectum	83.3[4] ± 3.0 a	82.0 ± 2.9 a	76.3[4] ± 3.5 b	78.2[3] ± 1.9 b	75.5[3] ± 1.2 b	77.2[3] ± 1.5 b	75.2[3] ± 2.7 b	76.9 ± 3.5 b
Feces	83.4** ± 1.8 a	83.5** ± 1.7 a	73.2 ± 2.4 d	79.6 ± 2.3 b	75.9 ± 2.1 cd	79.1* ± 2.2 b	77.9 ± 3.9 bc	80.9* ± 1.5 ab

[1] Mean ± $S.D._x$ for 5 pigs except as indicated by superscripts 3 and 4.

[2] Any means in the same row followed by the same letter did not differ significantly.

[3] Mean ± $S.D._x$ for 4 pigs.

[4] Mean ± $S.D._x$ for 3 pigs.

*For a given protein source values bearing one asterisk are different at $P<0.05$.

**For a given protein source values bearing two asterisks are different at $P<0.01$.

Table 31. True protein digestibility[1] of regular and low-fiber rapeseed meals, soybean meal and soybean isolate at various sites along the digestive tract of growing pigs.

	Protein Source							
			Rapeseed					
	Soybean		B.campestris		B. napus		B. napus cv. Bronowski	
Source of Digesta	Meal	Isolate	Meal	Low Fiber	Meal	Low Fiber	Meal	Low Fiber
Stomach	22.6[2] ±11.6	18.7 ±11.5	26.0 ± 7.2	21.5 ±11.0	21.9 ±12.6	30.8 ±15.4	21.9 ±18.0	26.6 ±21.9
	a[3]	a	a	a	a	a	a	a
Mid jejunum	72.6 ± 5.8	73.5 ± 8.3	74.3 ± 7.0	75.7 ± 5.6	76.9 ± 5.0	62.1 ±13.2	64.2 ±11.1	71.0 ± 6.4
	a	a	a	a	a	a	a	a
Ileum	81.0 ± 4.7	76.7 ± 6.8	80.1 ± 2.4	82.7[4] ± 3.2	80.8[4] ± 4.5	73.4[4] ±15.6	80.9 ± 5.5	76.2 ± 8.3
	a	a	a	a	a	a	a	a
Caecum	88.3 ± 2.3	90.5 ± 2.5	80.3 ± 1.7	83.7 ± 2.6	81.5 ± 2.9	80.6 ± 6.0	79.7 ± 2.3	78.4 ± 4.1
	a	a	bc	b	bc	bc	bc	c
Rectum	91.9[5] ± 3.0	91.2 ± 2.9	85.0[5] ± 3.5	86.8[4] ± 1.9	84.7[4] ± 1.2	85.9[4] ± 1.5	83.25[4] ± 2.7	85.4 ± 3.5
	a	a	b	b	b	b	b	b
Feces	90.2 ± 1.8	90.7 ± 1.7	80.0 ± 2.4	86.3 ± 2.3	83.2 ± 2.1	85.9 ± 2.2	84.3 ± 3.9	87.5 ± 1.5
	ab	a	e	cd	de	cd	d	bc

[1]Metabolic nitrogen in the digesta and feces for calculation of true digestibility coefficients was based on the nitrogen/Cr_2O_3 ratios in various segments of the alimentary tract of 3 pigs fed a protein-free diet in Experiment II.

[2]Mean ± $S.D._x$ for 5 pigs except as indicated by superscripts 4 and 5.

[3]Any mean in the same row followed by the same letter did not differ significantly ($P<0.05$).

[4]Mean ± $S.D._x$ for 4 pigs.

[5]Mean ± $S.D._x$ for 3 pigs.

Table 32. Dry matter digestibility (%) of regular and low-fiber rapeseed meals, soybean meal and soybean isolate at various sites along the digestive tract of growing pigs.

	Protein Source							
			Rapeseed					
	Soybean		B. campestris		B. napus		B. napus cv. Bronowski	
Source of Digesta	Meal	Isolate	Meal	Low Fiber	Meal	Low Fiber	Meal	Low Fiber
Stomach	30.0[1] ±13.5 a[2]	26.0 ±10.7 a	22.3 ± 9.5 a	22.8 ± 9.5 a	21.3 ±14.2 a	26.8 ±14.4 a	26.0 ±13.2 a	27.6 ±18.0 a
Mid jejunum	68.5 ± 2.1 ab	74.0 ± 2.0 a	57.6 ± 9.2 c	61.8 ± 3.2 bc	63.1 ± 6.1 bc	58.1 ± 7.9 c	57.9 ± 7.5 c	62.2 ± 6.6 bc
Ileum	76.9 ± 3.1 ab	78.7 ± 3.6 a	62.8 ± 6.2 d	68.4 ± 2.5 bcd	63.9 ± 4.8 d	69.1 ± 5.9 bcd	73.6 ± 5.2 abc	66.5 ±10.6 cd
Caecum	83.5 ± 3.5 a	84.7 ± 1.7 a	72.2 ± 4.0 cd	77.4 ± 3.2 b	74.0 ± 2.5 bcd	75.8 ± 3.3 bc	73.6 ± 3.4 bcd	70.2 ± 3.7 d
Rectum	86.1[4] ± 1.9 a	86.3 ± 2.5 a	80.1[4] ± 2.9 b	79.8[3] ± 1.9 b	77.6[3] ± 2.1 b	81.2[3] ± 1.8 b	80.3[3] ± 3.2 b	79.0 ± 4.5 b
Feces	86.0 ± 2.6 a	84.3 ± 2.1 a	75.3 ± 1.9 c	80.0 ± 1.8 b	78.4 ± 3.4 b	81.1 ± 0.4 b	80.5 ± 1.7 b	80.2 ± 2.4 b

[1] Mean ± $S.D._x$ for 5 pigs except as indicated by superscripts 3 and 4.

[2] Any mean in the same row followed by the same letter did not differ significantly ($P<0.05$).

[3] Mean ± $S.D._x$ for 4 pigs.

[4] Mean ± $S.D._x$ for 3 pigs.

tended to be higher for the "accepts" (air-classified low-hull) fractions of rapeseed meal as compared to the regular meal. Whether the improvement in protein digestibility particularly with soybean protein and low-hull (low-fiber) rapeseed meal is the result of microbial fermentation of protein in the caecum and large intestine or other factors such as differential transit of Cr_2O_3 is difficult to ascertain. The limitation of Cr_2O_3 as an index for digestibility determination for digesta is further indicated by the protein and dry matter digestibility data based on stomach contents (Table 32). These results indicate an appreciable concentration of the index material in digesta from the stomach even though no absorption is known to take place directly from this region of the alimentary tract. Twombly and Meyer (1961) also found that transit of Cr_2O_3 lags behind that of the digesta. This separation of index and digesta could result in an underestimation of digestibility on the basis of digesta taken from the small intestine. However Cr_2O_3 appears to be a satisfactory index for dry matter digestibility at various regions of the alimentary tract. The pattern of dry matter digestibility in the ileum, caecum and rectum was consistent to that found in the feces irrespective of protein source. However, whether the higher apparent or true digestibility of the air-classified (low-hull) fraction of rapeseed meal is a reflection of greater availability of this protein to the animal or the consequence of fermentation remains open to question. Fermentation might be more pronounced for protein when dry matter is relatively well digested as in the case of the soybean diets. In other words the presence of a lower amount of non-protein material in the large intestine of pigs fed soybean protein could augment fermentation of protein, thereby markedly improving the apparent digestibility

of the dietary protein. Thus the presence of a greater proportion of fibrous material in the digesta of pigs fed rapeseed meal, particularly the high-fiber regular meal, could depress fermentation of protein in the large intestine. Such a postulate is consistent with the fact that protein digestibility of high-fiber RSMs did not improve as much as that of the low-hull fraction from the ileum to the feces. It appears that the lower digestibility of RSM is primarily related to the fiber content of the diet rather than its poor absorption in the small intestine.

4.3.2 Total protein, soluble protein and amino nitrogen.

Distribution of the nitrogen in digesta from the various segments of the intestinal tract (Table 33) was consistent with previous observations for soybean protein and rapeseed meal (Experiment II). There were no appreciable differences among treatments in total protein, soluble protein and amino nitrogen in the digesta from the stomach of pigs fed the soybean and rapeseed diets. Protein content of digesta from the mid jejunum was significantly ($P<0.05$) higher than the protein content of digesta from the stomach for pigs fed the soybean protein diets whereas the protein content of digesta from the mid jejunum did not differ from that of the stomach for pigs fed rapeseed meal diets. On the other hand the proportion of soluble protein increased significantly ($P<0.01$) from the stomach to the mid jejunum for both protein sources although the proportion of soluble protein was higher ($P<0.01$) for pigs fed the soybean diets than for those fed the rapeseed diets. Amino nitrogen increased significantly ($P<0.01$) from the stomach to the mid jejunum for pigs fed both protein sources. The proportion of amino nitrogen in digesta from the mid jejunum also was similar for all dietary groups. However, the absolute amount of amino nitrogen (i.e. total

Table 33. Total protein, soluble protein and amino nitrogen in digesta from pigs fed regular and low-fiber rapeseed meals, soybean meal and soybean isolate[1].

Source of Digesta	Nitrogen Fraction	Protein Source: Soybean Meal[2]	Soybean Isolate[3]	Rapeseed: B. campestris Meal	B. campestris Low Fiber	B. napus Meal	B. napus Low Fiber	B. napus cv. Bronowski Meal	B. napus cv. Bronowski Low Fiber
Diet	Total Protein[4]	16.6	15.5	17.8	16.9	17.4	18.0	17.6	16.9
Stomach	Total Protein[5]	21.0[6] ± 1.6	20.3 ± 1.5	17.8 ± 0.9	19.0 ± 0.5	17.4 ± 0.1	17.7 ± 0.7	20.9 ± 0.9	19.4 ± 0.7
	Soluble Protein[7]	26.7 ± 5.8	34.3 ±11.8	34.5 ± 9.4	37.5 ± 6.7	31.8 ± 6.2	36.5 ± 9.2	32.3 ± 6.7	32.4 ± 8.6
	Amino Nitrogen[8]	7.7 ± 2.2	4.3 ± 1.9	6.2 ± 2.4	6.4 ± 1.5	7.4 ± 2.4	7.8 ± 1.5	7.8 ± 1.9	7.6 ± 2.2
Mid Jejunum	Total Protein	25.1 ± 1.7	28.5 ± 3.0	18.2 ± 3.1	19.5 ± 1.3	19.2 ± 4.3	23.0 ± 3.1	23.0 ± 2.7	22.1 ± 3.3
	Soluble Protein	89.3 ± 8.9	92.6 ± 4.3	72.4 ± 5.1	77.9 ± 6.3	75.2 ± 9.5	67.3 ± 8.9	69.9 ± 9.3	75.5 ± 4.5
	Amino Nitrogen	29.3 ± 7.0	21.6 ± 6.7	24.4 ± 6.2	26.5 ± 3.9	25.8 ± 5.0	26.2 ± 5.8	23.2 ± 8.7	25.8 ± 5.0
Ileum	Total Protein	21.1 ± 1.6	24.8 ± 0.9	13.5 ± 1.9	14.6[9] ± 2.5	12.9[9] ± 1.7	19.1[9] ± 4.6	19.6 ± 4.1	17.4 ± 2.2
	Soluble Protein	89.4 ± 5.1	88.2 ± 3.6	58.6 ± 4.6	64.0[9] ± 9.7	72.5[9] ± 9.6	60.2[9] ±11.3	69.4 ±10.7	78.8 ± 7.1
	Amino Nitrogen	31.1 ± 4.0	17.5 ± 4.9	23.5 ± 4.9	20.9[9] ± 2.8	16.2[9] ± 0.6	28.9[9] ± 8.1	18.6 ± 7.2	25.9 ±12.9
Caecum	Total Protein	20.4 ± 3.2	18.5 ± 1.4	16.7 ± 1.7	17.9 ± 1.7	16.2 ± 1.6	18.7 ± 2.8	18.9 ± 2.7	17.0 ± 3.2
	Soluble Protein	56.2 ±18.5	72.7 ± 5.6	35.2 ± 9.4	42.1 ± 9.8	35.2 ± 6.9	45.7 ± 2.7	27.4 ± 6.1	44.2 ± 7.0
	Amino Nitrogen	30.9 ± 5.9	18.8 ± 3.6	21.5 ± 3.4	29.7 ± 7.5	22.9 ± 4.6	23.4 ±12.4	24.9 ± 6.8	24.4 ± 0.7
Rectum	Total Protein	20.5[10] ± 2.2	20.7 ± 3.9	19.0[10] ± 1.1	17.7[9] ± 1.3	17.0[9] ± 1.9	19.9[9] ± 2.2	22.5[9] ± 2.0	18.7 ± 2.1
Feces	Total Protein	20.0 ± 2.6	16.4 ± 1.5	17.8 ± 1.3	16.9 ± 0.5	17.4 ± 2.1	18.0 ± 1.7	21.7 ± 4.5	20.0 ± 3.3
	Soluble Protein	36.3 ± 6.5	58.5 ±18.6	26.1 ± 2.4	26.1 ±11.1	25.5 ± 4.8	29.2 ± 7.1	21.6 ± 3.3	24.2 ± 2.5

[1] Statistical analysis of the data is given in Appendix, Table 2.

[2] 44% Protein.

[3] Isolated soybean protein (Nutritional Biochemical Co., Cleveland, Ohio).

[4] Expressed as % of dry matter of diet.

[5] Expressed as % of dry matter of digesta.

[6] All values are mean ± $S.D._{\bar{x}}$ for 5 pigs except as indicated by superscripts 9 and 10.

[7] Expressed as % of total protein of digesta.

[8] Amino nitrogen determined by formol titration and expressed as % of soluble protein using glycine as a reference.

[9] Mean ± $S.D._{\bar{x}}$ for 4 pigs.

[10] Mean ± $S.D._{\bar{x}}$ for 3 pigs.

protein x % soluble protein x % amino protein) was significantly ($P<0.05$) higher in digesta of pigs fed the soybean protein diets. Total protein content tended to decrease from the mid jejunum to the ileum for all groups although the decrease was more pronounced for pigs fed both regular and air-classified B. campestris rapeseed meals and the regular B. napus meal. Soluble protein and amino nitrogen in the ileum followed a similar pattern to that in the mid jejunum.

There was no consistent pattern in the total protein content among feces and digesta from the ileum and caecum. For example, total protein content tended to increase from the ileum to the feces for pigs fed both the regular and air-classified B. campestris meals and the regular B. napus meal whereas it tended to decrease with the soybean isolate diet. On the other hand there was a significant ($P<0.05$) decrease in the percentage of soluble protein between the ileum and feces with all dietary groups. Nevertheless the proportion of soluble protein tended to be higher in feces of pigs fed the soybean diets than those fed the RSM diets. This observation corresponded to the fact that the proportion of soluble protein tended to be higher in the digesta from the small intestine and caecum of pigs fed the soybean diets.

None of the parameters such as total protein, soluble protein and amino nitrogen appear to explain the differences in digestibility among the dietary groups. However, the proportion of soluble protein in the digesta from the small intestine and caecum was generally lower for pigs fed the regular and air-classified rapeseed meals. The low solubility of the RSM in the gut could limit the amount of amino acids readily available to intestinal microflora thereby decreasing the extent of fermentation in the caecum and the large intestine. This is in

agreement with a previous observation (Tables 30 and 31) that there was no difference in protein digestibility between soybean and rapeseed meal diets on the basis of digesta taken from the ileum whereas the digestibility of RSMs was significantly ($P<0.05$) lower on the basis of fecal analysis.

4.3.3 Amino acid composition of the diets and digesta of pigs fed low and high fiber rapeseed meals.

The amino acid composition of the soybean and rapeseed diets is given in Table 34. There were only slight differences in amino acid composition between soybean meal and soybean isolate. Similarly there were only minor differences in amino acid composition among the various rapeseed meals and removing the hull fraction had little effect on amino acid content. Composition of rapeseed meal, however, differed from that of soybean meal for several amino acids. The content of arginine, aspartic acid, serine, isoleucine, leucine, phenylalanine and tyrosine was lower for RSM than for soybean protein. By contrast RSM was higher in proline, glycine and methionine.

In general, levels of the individual amino acids in the regular rapeseed meals and the low-hull fractions of these meals all agreed within a range of 10%, which is the usual tolerance accepted in our laboratory for the amino acid composition of duplicate hydrolysates on the same sample. Recovery of nitrogen as ninhydrin-positive material during the chromatography of amino acids was 5 to 10% lower for B. campestris and B. napus rapeseed meals than for soybean protein. Nitrogen recovery in the case of the Bronowski meal did not differ appreciably from that of soybean meal. These results suggest the presence of a non-amino nitrogen fraction in B. campestris and B. napus rapeseed meals which could explain the lower recovery of nitrogen also found in the digesta and feces of pigs fed

Table 34. Amino acid composition (gm/16 gm N) of protein sources used in Experiment III.

	Protein Source							
			Rapeseed					
	Soybean		B. campestris		B. napus		B. napus cv. Bronowski	
Amino Acids	Meal[1]	Isolate[2]	Meal	Low Fiber[3]	Meal	Low Fiber[3]	Meal	Low Fiber[3]
Lysine	5.45	5.84	5.09	5.05	5.32	5.17	5.03	5.62
Histidine	2.14	2.38	2.34	2.25	2.35	2.53	2.76	2.74
Arginine	6.58	7.96	5.15	5.53	5.80	5.78	6.12	6.38
Aspartic Acid	11.70	13.31	6.49	6.90	6.42	6.54	6.95	7.10
Threonine	3.98	4.04	3.87	4.24	4.16	4.05	4.36	4.40
Serine	5.37	5.55	3.96	4.21	4.17	4.26	4.54	4.62
Glutamic Acid	19.60	21.91	16.91	18.13	19.38	18.71	18.31	20.33
Proline	5.21	5.20	6.14	6.61	6.55	6.15	6.47	6.68
Glycine	4.27	4.39	4.75	4.93	5.18	4.82	5.15	5.24
Alanine	4.27	4.26	4.24	4.32	4.13	4.38	4.63	4.76
Cystine	1.04	0.69	0.83	0.53	1.14	1.33	1.20	1.45
Valine	4.37	4.72	4.07	4.44	4.08	4.51	4.94	4.90
Methionine	1.54	1.30	1.90	2.16	1.56	1.65	2.00	1.80
Isoleucine	4.25	4.79	2.89	3.14	3.21	3.37	3.93	3.67
Leucine	7.91	9.37	6.62	7.02	5.94	7.03	7.68	7.64
Tyrosine	3.25	3.91	2.42	2.62	2.65	2.56	2.47	2.79
Phenylalanine	4.87	5.65	3.74	3.77	3.72	3.90	3.99	4.24
% Protein in Diets	16.62	15.50	16.37	16.58	15.40	16.30	17.64	16.85
% Nitrogen Recovery[4]	92.5	97.0	80.5	83.6	84.4	86.6	90.7	94.1

[1] 44% Protein

[2] Alpha Protein, Nutritional Biochemicals Co., Cleveland, Ohio.

[3] Seed hull removed by air-classification (Bower Bros., N.Y.)

[4] % Recovery = $\frac{\text{N in Ninhydrin - positive Compounds (including } NH_3\text{)} \times 100}{\text{Total N Applied to Column}}$

these meals (Tables 20 and 35).

In general, the amino acid composition of the dietary proteins was reflected in the amino acid patterns of the digesta and feces (Table 35). Except for histidine, arginine and glutamic acid, amino acid composition of the feces bore a closer relationship with that of the diet than did amino acid composition of digesta from the ileum and mid jejunum. For example, the higher content of aspartic acid in soybean protein was reflected by a significantly ($P<0.01$) higher content of this amino acid in the feces of pigs fed either the meal or isolate compared to that of pigs fed rapeseed protein. Similarly, the higher content of proline in rapeseed meal coincided with a significantly ($P<0.01$) higher level of this amino acid in the feces of pigs fed this protein.

Although the amino acid pattern of digesta in the mid jejunum did not vary appreciably from that of the diets there was a considerable increase in the proportion of proline and glycine and a decrease in the proportion of arginine, methionine, isoleucine, leucine, tyrosine and phenylalanine for all protein sources. The amino acid composition of digesta in the ileum of pigs fed the different dietary protein sources was closely related to that in the mid jejunum. Several changes occurred in the amino acid pattern of digesta between the ileum and rectum. There was a marked reduction in proline and glycine which corresponded to the results of the Experiment II. There was also a significant ($P<0.05$) decrease in the proportion of glutamic acid with soybean meal, soybean isolate and Bronowski meal but not with the B. campestris meal. On the other hand, the proportion of alanine, methionine and isoleucine in the feces was higher ($P<0.05$ to $P<0.01$)

Table 35. Amino acid composition of the diet, digesta and feces of pigs fed regular and low-fiber rapeseed meals, soybean meal and soybean isolate.

Dietary Regimen	Source of Digesta	Amino Acids (gm/16 gm N)[1]																	% Nitrogen Recovery[2] ± S.D.$_x$	Protein (%) ± S.D.$_x$
		Lys	His	Arg	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val	Met	Ile	Leu	Tyr	Phe		
Soybean - Meal	Diet	5.6	2.2	6.8	12.0	4.1	5.5	20.1	5.3	4.4	4.4	1.1	4.5	1.6	4.4	8.1	3.3	5.0	92.5	
	Mid jejunum	5.4[3]	2.0	5.1	12.1	4.1	5.2	18.0	10.1	8.6	4.6	1.7	4.2	1.1	3.6	6.2	2.8	3.8	87.7±5.2[3]	25.1±1.7[3]
	Ileum	6.9	1.9	5.0	12.8	4.6	4.9	22.1	15.7	9.1	4.1	1.3	3.3	0.8	2.5	5.1	2.2	3.0	77.5±2.4	21.0±1.5
	Feces	6.9	1.6	3.8*	11.1 ab	5.4*	4.9	13.7*	4.6* a	6.2*	7.8*	0.8	5.9*	2.9**	4.8*	8.7*	3.9*	4.9*	77.7±3.3	20.0±2.6
- Isolate	Diet	5.7	2.3	7.8	13.0	4.0	5.4	21.5	5.1	4.3	4.2	0.7	4.6	1.3	4.7	9.2	3.8	5.5	97.0	
	Mid jejunum	4.5[4]	1.8	4.5	14.0	4.1	6.1	18.9	12.3	10.0	3.7	0.1	3.1	0.7	2.8	4.6	2.0	2.9	92.8±4.5[4]	15.5
	Ileum	5.5[4]	2.0	4.1	18.1	5.2	8.1	22.2	8.5	7.0	3.4	0.2	3.1	0.8	2.9	4.6	2.1	3.0	78.9±3.5	28.5±3.0[4]
	Feces	5.8	1.7	4.1	12.6* a	5.4	6.6*	14.9*	4.5* a	5.1*	5.7*	0.5	4.8*	2.5**	4.3*	7.1*	3.2*	4.5*	78.5±2.7	25.0±0.9[4]
																				16.4±1.5*
B. campestris - Meal	Diet	6.0	2.8	6.1	7.7	4.6	4.7	20.0	7.2	5.6	5.0	1.0	4.8	2.2	3.4	7.8	2.9	4.4	80.5	
	Mid jejunum	5.6[4]	1.8	4.0	9.0	5.3	5.3	15.3	10.9	12.8	4.7	0.7	4.9	1.1	3.2	6.3	2.3	3.2	75.8±4.1[4]	16.4
	Ileum	6.1[4]	2.0	3.9	8.9	6.3	5.6	12.8	13.3	9.2	4.5	0.6	5.2	1.3	3.6	6.1	2.7	3.6	68.4±3.8[4]	18.2±3.1[4]
	Feces	6.3	1.3*	4.2	9.7 c	5.6*	5.0	12.8	6.7* b	5.8*	6.3*	0.4	5.6	2.7**	4.4*	7.7*	3.5*	4.6*	65.7±1.8	12.8±1.2*
																				17.8±1.3
- Low Fiber	Diet	5.7	2.6	6.3	7.8	4.8	4.8	20.6	7.5	5.6	4.9	0.6	5.0	2.5	3.6	8.0	3.0	4.3	83.6	
	Mid jejunum	6.1[4]	1.7	3.9	8.8	5.1	5.0	16.0	11.1	13.1	4.6	0.8	4.6	1.0	3.1	5.8	2.4	2.8	75.5±3.0[4]	16.5
	Ileum	6.8[4]	1.7	3.4	8.5	6.3	5.4	13.4	16.2	8.4	4.1	0.3	4.9	1.1	3.2	5.6	2.6	3.0	67.8±2.3[4]	19.3±1.3
	Feces	6.8	1.4	4.3	9.8 c	5.7	5.1	12.8	7.4* b	6.0*	6.4*	0.3	5.9*	2.9**	5.3*	8.1*	3.6*	4.5*	68.7±4.4	14.6±2.5[4]
																				16.9±0.5
B. napus- Meal cv. Bronowski	Diet	5.3	2.9	6.4	7.3	4.6	4.8	19.2	6.8	5.4	4.8	2.1	5.2	2.1	4.1	8.0	2.6	4.2	90.7	
	Mid jejunum	5.8	2.0	4.3	8.8	5.2	5.4	17.0	9.9	10.6	4.7	2.1	5.2	1.2	3.4	6.5	2.3	3.4	85.5±2.9	17.6
	Ileum	6.6	2.1	4.2	10.1	6.9	6.0	14.0	9.5	7.4	5.0	1.3	5.5	1.4	3.8	7.3	2.8	3.9	69.1±3.1	23.0±2.7
	Feces	7.1	1.7*	4.1	9.4 c	5.3*	4.9*	11.6*	6.4 b	5.6	6.1*	0.3	5.6	2.2**	4.7*	7.9*	3.2	4.2	70.3±2.9	19.6±4.1
																				20.0±3.3
- Low Fiber	Diet	5.7	2.8	6.4	7.2	4.4	4.7	20.5	6.7	5.3	4.8	1.5	5.0	1.8	3.7	7.7	2.8	4.3	94.1	
	Mid jejunum	5.5	1.9	4.6	7.8	4.3	4.4	15.6	12.7	11.0	4.9	2.1	4.5	1.2	3.1	6.1	2.2	3.0	82.5±3.3	16.9
	Ileum	6.4	1.5	4.3	9.0	5.6	5.0	15.3	16.8	9.1	4.7	1.2	4.6	1.2	3.3	6.0	2.2	3.2	69.1±4.2	22.1±3.3
	Feces	6.4	1.7	4.2	10.1 bc	5.6	5.1	13.1*	6.6* b	6.0*	6.5*	0.5	6.0*	2.3**	4.7*	8.0*	3.7*	4.5*	71.7±4.6	17.4±2.2
																				16.4±2.2

[1]Amino acid percentage values were corrected to 95% recovery of amino acids as recommended by Knipfel et al., 1971.

[2]Recovery of nitrogen was calculated by summation of nitrogen contained in the ninhydrin-positive material (including ammonia) relative to the total N applied to the column during ion exchange chromatography of amino acids.

[3]Each value for digesta from the mid jejunum, ileum and feces represents the mean (± S.D.$_x$) of 5 observations except as indicated by superscript 4.

[4]Mean (± S.D.$_x$) for 4 observations.

*Any value from the feces with one asterisk is significantly ($P < 0.05$) different from that of the ileum for a given protein source.

**Any value from the feces with two asterisks is significantly ($P < 0.01$) different from that of the ileum for a given protein source.

[a,b,c]Values from the feces for a particular amino acid and which are not followed by the same letter differ significantly ($P < 0.01$).

than in digesta from the ileum and mid jejunum with all dietary treatments. Similarly, there was an overall significant ($P<0.05$) increase in the proportion of leucine, tyrosine and phenylalanine, except with the regular Bronowski meal. However, there were no marked changes between the ileum and feces in the proportion of lysine, histidine, arginine, aspartic acid, threonine and serine for pigs irrespective of diets.

Removal of the hull fraction from rapeseed meal had no apparent effect on the amino acid pattern of digesta and feces. Similarly there were no differences in the amino acid patterns of digesta and feces of pigs fed soybean meal or soybean isolate. Furthermore the presence of the hull fraction in RSM did not affect the amino acid pattern of digesta during transit through the large intestine.

Except for proline and glycine, dilution of digesta by endogenous proteins was insufficient to mask the amino acid composition of the diets. This was particularly obvious for aspartic acid where the higher content of this amino acid in the digesta of pigs fed soybean protein reflected the higher proportion of this amino acid in the diet. However dilution by endogenous protein was difficult to ascertain because of the relative similarity in the amino acid pattern of soybean and rapeseed protein. Nevertheless, these results are consistent with those of Experiment II where endogenous protein did not mask the amino acid composition of the diet. Furthermore Cho and Bayley (1972) reported a two-fold increase in the concentration of nitrogen in the duodenum which like the present results contradict the proposal by Nasset and co-workers (1955, 1957, 1961, 1963) that extensive dilution of dietary proteins occurs in the small intestine.

The close relationship between dietary amino acids and amino acid pattern of feces in the present experiment suggests that reabsorption of endogenous protein is nearly complete by the time digesta reaches the large intestine or that differential degradation of proline occurred in the large intestine. The possibility of microbial modification of the unabsorbed protein residues as the digesta passed through the large intestine appears a more plausible explanation to the differences in amino acid pattern of feces and digesta from the ileum. In fact, the amino acid composition of feces was very similar except for aspartic acid and proline with soybean and rapeseed protein. The significant ($P<0.05$ to $P<0.01$) differences observed in the proportions of glutamic acid, proline, glycine, alanine, methionine and isoleucine between feces and digesta from the ileum coincide with the observations reported by Cho and Bayley (1972). However results herein reported differ from those of these researchers in that there was a significantly ($P<0.05$) higher level (gm/16 gm N) of leucine, tyrosine and phenylalanine with most dietary treatments and no marked reduction in lysine for either protein source between the ileum and feces.

4.3.4 Amino acid digestibility of soybean and rapeseed proteins based on feces and digesta from the mid jejunum and ileum.

In general apparent and true digestibilities of the individual amino acids were higher for soybean meal and soybean isolate than for the rapeseed samples (Tables 36 and 37). With the exception of histidine and methionine, overall digestibilities of the individual amino acids were highest for soybean isolate and lowest for B. campestris meal. On the other hand, except for arginine, aspartic acid, serine, proline and isoleucine, apparent and true digestibilities of amino acids in the

Table 36. Apparent digestibility (%) of the individual amino acids of regular and low-fiber rapeseed meals, soybean meal and soybean isolate.

Amino Acids	Protein Source					
			Rapeseed			
	Soybean		B. campestris		B. napus cv. Bronowski	
	Meal	Isolate	Meal	Low Fiber	Meal	Low Fiber
Lysine	79.7[1]b[2]EFG[3]	83.4 a CDE	72.0 e CDE	75.8 cd BCD	72.8 de CDE	78.7 bc BCDE
Histidine	88.2 a ABC	87.8 a ABC	87.1 a A	88.5 a A	87.8 a A	88.9 a A
Arginine	90.5 a A	91.5 a A	81.6 c B	86.2 b A	86.2 b A	87.8 b A
Aspartic Acid	84.8 a CD	84.0 a BCDE	66.1 c G	74.7 b CDE	70.9 b E	73.1 b G
Threonine	77.9 a FG	78.2 a F	67.4 b FG	75.8 a BCD	74.8 a BCDE	76.1 a EFG
Serine	85.0 a BCD	79.9 b EF	71.2 c CDEF	78.3 b BCD	77.3 b BCD	79.0 b BCDE
Glutamic Acid	88.7 a AB	88.6 a AB	82.9 c B	87.4 ab A	86.4 b A	87.8 ab A
Proline	85.8 a BCD	85.4 a BC	75.1 c C	80.0 b B	79.1 b B	81.3 b B
Glycine	77.7 a G	80.5 a DEF	72.3 b CD	78.0 a BCD	77.3 a BCD	78.6 a BCDE
Alanine	70.4 bcH	77.5 a F	66.5 c G	73.6 ab DE	72.3 b DE	74.4 ab FG
Valine	78.2 b FG	83.6 a CDE	68.7 c DEFG	76.2 b BCD	75.5 bc BCDE	76.9 b DEF
Methionine	69.6 a H	68.0 a G	68.7 a DEFG	76.3 a BCD	76.8 a BCD	76.1 a EFG
Isoleucine	81.6 a DEFG	84.8 a BCD	65.7 c G	70.3 c E	75.2 b BCDE	75.6 b EFG
Leucine	82.2 b DEF	87.2 a ABC	73.6 c C	79.3 b BC	78.4 b B	80.2 b BCD
Tyrosine	80.4 b EFG	86.5 a BC	67.6 e EFG	75.6 cd BCD	72.7 d CDE	77.4 bc CDEF
Phenylalanine	83.8 abCDE	86.3 a BC	72.3 d CD	79.1 c BC	77.9 c BC	80.3 bc BC
Protein	83.4 ab	83.5 a	73.2 e	79.6 cd	77.9 d	80.9 bc

[1]Each value represents the mean of 5 observations based on fecal samples.

[2]Values in the same row followed by the same small letter did not differ significantly ($P<0.05$).

[3]Values in the same column followed by the same capital letter did not differ significantly ($P<0.05$).

Table 37. True digestibility (%) of the individual amino acids of regular and low-fiber rapeseed meals, soybean meal and soybean isolate.

Amino Acids	Protein Source					
	Soybean		Rapeseed			
			B. campestris		B. napus cv. Bronowski	
	Meal	Isolate	Meal	Low Fiber	Meal	Low Fiber
Lysine	88.1[1]b[2]DEF[3]	92.1 a ABCDE	79.9 d CD	84.0 c B	80.0 d D	86.8 bc B
Histidine	93.9 a AB	93.6 a AB	91.7 a A	93.5 a A	91.9 a A	93.4 a A
Arginine	94.8 a A	95.5 a A	86.4 c B	90.8 b A	90.5 b A	92.2 b A
Aspartic acid	90.7 a CDE	89.1 a DEF	75.6 c EF	83.8 b BC	80.1 b CD	82.9 b C
Threonine	87.7 abEFG	88.8 a EF	76.3 d DEF	84.1 c B	83.0 c BC	85.0 bc BC
Serine	91.4 a BCD	87.1 b F	79.2 c CDE	86.1 b B	84.5 b B	86.8 b B
Glutamic Acid	92.8 a ABC	92.6 a ABCDE	87.1 c B	91.3 abA	90.4 b A	91.7 ab A
Proline	90.2 a CDEF	90.3 a BCDE	78.4 c CDEF	83.1 b C	82.4 b BCD	84.7 b BC
Glycine	84.2 b GH	89.1 a DEF	78.6 c CDEF	84.1 b B	83.4 b BC	85.1 b BC
Alanine	81.1 bcH	89.6 a CDEF	76.0 c DEF	83.2 b B	81.4 bcBCD	84.0 b BC
Valine	86.8 b FG	92.2 a ABCDE	76.8 d DEF	83.8 bcB	82.5 c BCD	84.5 bc BC
Methionine	81.0 a H	83.1 a G	76.8 a DEF	83.7 a B	84.9 a B	85.9 a BC
Isoleucine	89.0 a DEF	92.2 a BCD	75.2 d F	79.2 c C	82.6 bcBCD	84.1 b BC
Leucine	88.4 b DEF	93.1 a ABC	80.1 d C	85.7 bcB	84.3 c BC	86.9 bc B
Tyrosine	87.1 b EFG	92.8 a ABCD	75.5 e EF	83.1 cdC	80.8 d BCD	85.2 bc BC
Phenylalanine	89.2 abCDEF	91.8 a ABCDE	78.5 d CDEF	85.4 bcB	83.9 c BCD	86.5 bc B
Protein	90.2 ab	90.8 a	80.0 e	86.4 cd	84.3 d	87.5 bc

[1]Each value represents the mean of 5 observations based on fecal samples.

[2]Values in the same row followed by the same small letter did not differ significantly ($P < 0.05$).

[3]Values in the same column followed by the same capital letter did not differ significantly ($P < 0.05$).

low-fiber Bronowski meal did not differ significantly ($P<0.05$) from those found for soybean meal. Apparent digestibility of methionine tended to be lower for the soybean samples than for the rapeseed samples but true digestibility of methionine did not differ between these two protein sources.

Fiber tended to depress protein and amino acid digestibilities. In fact fiber had a significant effect on the digestibility of all amino acids except histidine, serine, and methionine. The higher overall digestibilities of amino acids for the low-fiber diets was primarily due to the appreciably higher digestibilities of amino acids with the low-fiber B. campestris meal than with the regular B. campestris meal. Fiber had only a slight effect on the digestibilities of amino acids of soybean and Bronowski rapeseed although there was a small but significant ($P<0.05$) difference in protein digestibility between the regular and low-fiber Bronowski samples. Of interest, however, was the significantly ($P<0.05$) higher digestibility of lysine and tyrosine with the low-fiber Bronowski meal. Apparent and true digestibility of 10 of the 16 amino acids followed a similar pattern for both soybean meal and soybean isolate. The digestibilities of lysine, alanine, valine, leucine, and tyrosine were significantly higher ($P<0.05$) for soybean isolate than soybean meal while serine was the only amino acid for which digestibility was significantly ($P<0.05$) higher for the meal than the isolate.

Although fiber had a marked effect on the overall digestibilities of the individual amino acids, it had no apparent effect on the relative digestibilities within the various protein sources (Tables 36 and 37). Nevertheless there were some interesting observations with respect to the relative digestibilities of individual amino acids within protein

sources. The apparent and true digestibilities of histidine, arginine, and glutamic acid were consistently higher than those of the other amino acids for both soybean and rapeseed proteins. In addition, the digestibility of methionine, threonine, glycine and alanine tended to be lower than that of the other amino acids for soybean meal and soybean isolate but the same pattern did not prevail for the rapeseed samples. In fact, except for the higher digestibility of histidine, arginine, and glutamic acid there was no consistent pattern in the relative digestibility of individual amino acids with the various RSM samples.

Apparent and true digestibilities of amino acids based on fecal analyses are compared with the digestibility coefficients based on digesta from the mid jejunum and ileum in Tables 38 and 39. Statistical analysis indicated that the digestibility coefficients for histidine, glycine, threonine and serine were significantly ($P<0.05$) higher on the basis of fecal analysis than digestibilities based on digesta from the ileum for all protein sources. Similarly there was a marked ($P<0.05$) improvement in digestibility between the ileum and feces for arginine, aspartic acid and glutamic acid with the soybean proteins and the Bronowski rapeseed samples but not with the B. campestris samples. On the other hand, digestibility of lysine was significantly ($P<0.05$) higher in the feces than the ileum only for the soybean proteins and the low-fiber Bronowski meal. There were no differences between the ileum and feces in the digestibilities of tyrosine and isoleucine. Similarly, except for the low-fiber Bronowski rapeseed meal, there were no significant ($P<0.05$) improvements in digestibilities for alanine, valine and leucine between the ileum and feces. By contrast, the apparent digestibility of methionine was significantly ($P<0.05$) lower

Table 38. Apparent digestibility[1] (%) of the individual amino acids of regular and low-fiber rapeseed meals, soybean meal and soybean isolate at various sites along the intestinal tract of growing pigs.

Dietary Regimen	Source of Digesta	Amino Acids																Protein Dig. (%) ± S.D.$_x$
		Lys	His	Arg	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe	
Soybean - Meal	Mid jejunum	53.8[2]	55.7	64.0	52.2	51.9	55.3	57.5	8.7	6.1	50.4	55.7	68.4	61.2	63.9	60.0	64.2	52.3± 5.8[2]
	Ileum	64.2	72.9	82.6	68.8	66.8	73.4 a	67.9	21.0 a	38.9	72.6	78.6	84.2	83.5	81.7	80.6	82.8	70.8± 4.7
	Feces	79.7*	88.2*	90.5*	84.8*	77.9*	85.0*	88.7*	85.8*	77.7*	70.4	78.2	69.6*	81.6	82.2	80.4	83.8	83.4± 1.8*
- Isolate	Mid jejunum	60.8[3]	62.2	73.7	48.5	49.5	46.6	57.8	-18.8	-13.0	57.5	67.6	69.4	71.0	75.6	74.8	74.7	51.8± 8.3[3]
	Ileum	66.5[3]	71.4	81.5	53.0	55.4	50.1 a	64.2	42.0 a	42.4	72.4	80.0	78.8	79.3	83.0	81.1	81.7	66.1± 7.9[3]
	Feces	83.4*	87.8*	91.5*	84.0*	78.2*	79.9*	88.6*	85.4*	80.5*	77.5	83.6	68.0*	84.8	87.2	86.5	86.3	83.5± 1.7*
B. campestris - Meal	Mid jejunum	55.9[3]	71.1	69.1	46.0	47.8	47.5	64.3	31.7	17.7	57.5	53.7	77.9	56.5	63.3	62.7	66.2	53.8± 7.0[3]
	Ileum	67.8[3]	77.4	79.9 b	63.5	57.2 b	62.3 b	79.8	42.2	48.1	72.1	66.1 b	82.3	66.9 b	75.4 b	70.4	77.1	68.9± 1.6[3]
	Feces	72.0	87.1*	81.6	66.1	67.4*	71.2*	82.9	75.1*	72.3*	66.5	68.7	68.7*	65.7	73.6	67.6	72.3*	73.2± 2.4
- Low Fiber	Mid jejunum	52.5[3]	71.3	72.0	49.9	52.7	53.2	65.4	34.5	- 8.4	58.4	59.1	82.3	61.4	67.8	63.8	70.6	55.5± 5.6[3]
	Ileum	67.5[3]	81.4	85.0 b	70.1	63.9 b	70.0 b	81.9	42.4	58.6	77.0	73.2 b	87.8	75.1 b	80.7 b	76.0	80.6	72.5± 3.2[3]
	Feces	75.8	88.5*	86.2	74.7	75.8*	78.3*	87.4	80.0*	78.0*	73.6	76.2	76.3*	70.3	79.3	75.6	79.1	79.6± 2.3
B. napus- Meal cv. Bronowski	Mid jejunum	42.4	62.7	63.9	34.0	37.0	37.4	51.5	19.2	- 7.0	46.7	44.3	70.8	54.8	53.2	50.4	56.1	45.2±11.1
	Ileum	63.3	79.0	81.4	60.6	56.5	64.1	76.8	59.9 c	59.9	70.4	69.7	81.0	73.7	74.1	68.8	73.3	72.3± 5.1
	Feces	72.8	87.8*	86.2*	70.9*	74.8*	77.3*	86.4*	79.1*	77.3*	72.3	75.5*	76.8	75.2	78.4	72.7	77.9*	77.9± 3.9
- Low Fiber	Mid jejunum	52.4	66.7	65.2	44.3	52.4	53.0	62.4	16.0	8.8	49.0	55.0	66.5	58.5	60.2	60.7	65.6	51.2± 6.5
	Ileum	62.2	78.1	78.0	57.8	58.3	64.3	74.9	35.6 c	39.4	66.5	68.9	78.2	70.4	74.0	73.8	74.9	66.1± 8.3
	Feces	78.7*	88.9*	87.8*	73.1*	76.1*	79.0*	87.8*	81.3*	78.6*	74.4*	76.9*	76.1	75.6	80.2*	77.4	80.3*	80.9± 1.5*

[1]All apparent digestibility coefficients of amino acids are corrected to 95% recovery of amino acids (Knipfel et al., 1971).

[2]Each digestibility value represents the mean (± S.D.$_x$) of 5 observations except as indicated by superscript 3.

[3]Mean (± S.D.$_x$) of 4 observations.

*Digestibility values from the feces with an asterisk are significantly ($P < 0.05$) different from those of the ileum.

[a,b,c]Any digestibility values of the digesta from the ileum bearing the same letter for a given protein source (soybean protein, B. campestris and B. napus cv. Bronowski) and a given amino acid are significantly ($P < 0.05$) different.

Table 39. True digestibility[1] (%) of the individual amino acids of regular and low-fiber rapeseed meals, soybean meal and soybean isolate at various sites along the intestinal tract of growing pigs.

Dietary Regimen	Source of Digesta	Amino Acids																Protein ± S.D.$_x$
		Lys	His	Arg	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe	
Soybean - Meal	Mid jejunum	65.0[2]	65.6	75.9	60.8	69.0	69.2	63.7	107.0	82.2	67.9	69.8	76.8	68.0	72.4	69.6	71.0	72.6±5.8[2]
	Ileum	68.8	81.3	88.0	74.4	79.9	82.1	72.1	68.0	78.3	81.5	86.2	88.5	88.2	86.8	86.2	87.1	81.0±4.7
	Feces	88.1	93.9	94.8	90.7	87.7	91.4	92.8	90.2	84.2	81.1	86.8	81.0	89.0	88.4	87.1	89.2	90.2±1.8
- Isolate	Mid jejunum	70.7[3]	72.1	82.9	57.0	68.5	61.8	63.9	85.9	70.2	77.3	82.3	80.6	77.8	83.7	83.7	81.4	73.5±8.3[3]
	Ileum	71.7[3]	78.4	86.5	58.4	69.8	59.5	68.3	102.9	85.2	82.4	84.7	84.4	83.9	87.7	86.1	85.9	76.5±7.9[3]
	Feces	92.1	93.6	95.5	89.1	88.8	87.1	92.6	90.3	89.1	89.6	92.2	83.1	92.2	93.1	92.8	91.8	90.8±1.7
B.campestris - Meal	Mid jejunum	64.8[3]	79.0	82.6	59.7	65.6	64.3	70.6	105.4	56.1	73.1	67.1	83.9	65.4	72.3	74.2	75.0	74.3±7.0[3]
	Ileum	72.8[3]	83.2	86.3	72.6	69.2	73.0	84.2	83.8	79.9	80.3	73.5	85.5	73.2	80.9	77.1	82.2	79.3±1.6[3]
	Feces	79.9	91.7	86.4	75.6	76.3	79.2	87.1	78.4	78.6	76.0	76.8	76.8	75.2	80.1	75.5	78.5	80.0±2.4
- Low Fiber	Mid jejunum	61.8[3]	79.8	84.8	63.1	67.3	69.3	71.4	104.7	56.0	74.2	71.7	87.7	67.6	76.5	74.6	78.6	75.7±5.6[3]
	Ileum	72.3[3]	87.4	90.8	78.5	74.9	79.9	85.9	81.0	89.3	84.9	79.9	90.5	80.8	85.8	82.0	85.7	82.7±3.2[3]
	Feces	84.0	93.5	90.8	83.8	84.1	86.1	91.3	83.1	84.1	83.2	83.8	83.7	79.2	85.7	83.1	85.4	86.4±2.3
B. napus- Meal cv. Bronowski	Mid jejunum	51.6	69.4	76.0	47.4	51.5	52.5	57.3	91.6	55.3	61.4	55.2	77.0	61.5	63.5	61.7	63.8	64.2±11.1
	Ileum	68.2	84.0	86.7	69.1	67.4	73.5	80.8	100.1	89.9	77.8	75.8	84.0	78.3	78.8	75.1	78.1	80.9±5.5
	Feces	81.1	91.9	90.5	80.1	83.0	84.5	90.4	82.4	83.4	81.4	82.5	84.9	82.6	84.3	80.8	83.9	84.3±3.9
- Low Fiber	Mid jejunum	61.6	74.4	77.4	60.5	68.0	69.4	68.3	84.3	59.4	64.8	68.3	73.7	66.4	69.0	71.9	73.5	71.2±6.5
	Ileum	66.9	83.6	83.5	66.9	70.1	74.3	78.9	60.9	71.4	74.4	75.6	81.8	75.8	79.2	80.1	79.8	76.2±8.3
	Feces	86.8	93.4	92.2	82.9	85.0	86.8	91.7	84.7	85.1	84.0	84.5	85.9	84.1	86.7	85.2	86.5	87.5±1.5

[1]All true digestibility coefficients of amino acids are corrected to 95% recovery of amino acids (Knipfel et al., 1971).

[2]Each digestibility value represents the mean (± S.D.$_x$) of 5 observations except as indicated by superscript 3.

[3]Mean (± S.D.$_x$) of 4 observations.

in the feces than in the ileum with the soybean protein and the B. campestris meals (Table 38). However, true digestibility of methionine (Table 39) did not differ appreciably between the ileum and feces.

Digestibility coefficients based on digesta from the ileum did not differ appreciably among protein sources for many of the amino acids. These observations are in contrast to the data based on fecal analyses where digestibilities of most amino acids were significantly ($P<0.05$) higher for soybean protein than for rapeseed protein (Table 35). However, as in the case of fecal analyses, digestibility coefficients based on digesta from the ileum were significantly ($P<0.05$) higher for isoleucine, tyrosine and phenylalanine with soybean than with rapeseed meal. Nevertheless, overall digestibilities of the amino acids in the ileum were not consistently high for soybean isolate and low for the regular B. campestris as was the case with feces. Furthermore apparent and true digestibilities of histidine, arginine, isoleucine, leucine, tyrosine and phenylalanine tended to be higher than that of lysine, aspartic acid, threonine, serine, glutamic acid, and alanine for the ileum whereas in the feces only the digestibilities of histidine, arginine, and glutamic acid were consistently higher than those of the other amino acids for all protein sources.

As in the case of feces, fiber tended to depress the digestibility of amino acids in the ileum of pigs fed regular B. campestris meal. In fact, the digestibility of arginine, threonine, serine, valine, isoleucine and leucine was significantly ($P<0.05$) higher for low-fiber B. campestris meal than for the regular B. campestris meal. On the other hand, fiber had no apparent effect on amino acid digestibilities with soybean protein and Bronowski rapeseed. These observations are

consistent with the lack of a significant effect of fiber on amino acid digestibilities based on fecal analysis.

Pattern of digestibility in the mid jejunum was similar to that in the ileum although there was a marked improvement in digestibility between the mid jejunum and ileum. Apparent and true digestibilities of arginine, methionine, histidine, leucine, tyrosine, and phenylalanine were consistently higher than the digestibilities of lysine, aspartic acid, threonine, serine, and glutamic acid in both the mid jejunum and ileum. THe similarity in pattern of amino acid digestibility between the ileum and mid jejunum coincides with the fact that there were no appreciable differences in amino acid composition in these regions of the intestinal tract.

In general, results on amino acid digestibilities for the present experiment agree with the results for isolated soybean and regular rapeseed meals in Experiment II (Table 40). There was a close agreement between the two experiments in the pattern of digestion of soybean isolate. In both experiments there was a marked improvement in the digestibilities of aspartic acid, threonine, and serine, together with an appreciable improvement in the digestibilities of lysine, histidine, arginine, glutamic acid, proline and glycine between the ileum and feces. In addition, digestibilities of alanine, isoleucine, leucine, tyrosine and phenylalanine did not improve between the ileum and feces of pigs fed soybean isolate. The only difference in digestibility pattern between the two experiments was with methionine. There was a significant ($P<0.05$) decrease in the apparent digestibility of methionine between the ileum and feces in Experiment III whereas no difference in digestibility was found between the ileum and feces in

Table 40. Comparison of apparent amino acid digestibility coefficients[1] of soybean isolate and rapeseed meal protein in ileum and feces of pigs during Experiments II and III.

Protein Source	Source of Digesta	Amino Acids																Protein Dig.(% ±S.D.x
		Lys	His	Arg	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe	
Soy-Isolate[2] (Exp. II)	Ileum	75.8	78.9	85.6	57.1	51.8	51.4	73.8	55.2	40.3	73.6	77.3	61.2	76.4	82.2	82.0	80.7	70.3±3.
	Feces	84.8	89.2	92.8	84.0	72.2	81.0	88.6	86.2	80.0	77.0	82.3	66.5	84.8	88.2	86.2	88.1	84.1±1.
Soy-Isolate (Exp. III)	Ileum	66.5	71.4	81.5	53.0	55.4	50.1	64.2	42.0	42.4	72.4	80.6	78.8	79.3	83.0	81.1	81.7	66.1±7.
	Feces	83.4	87.8	91.5	84.0	78.2	79.9	88.6	85.4	80.5	77.5	83.6	68.0	84.8	87.2	86.5	83.6	83.5±1.
RSM-5[3] (Exp. II)	Ileum	72.5	84.5	88.3	73.9	71.5	76.0	85.6	49.8	67.3	79.8	77.7	86.9	77.6	79.9	81.2	79.7	77.2±5.
	Feces	69.5	84.0	82.9	68.4	70.9	74.6	84.8	78.9	74.2	70.6	73.1	71.3	69.8	74.8	74.9	74.7	74.2±1.
B.campestris[4] (Exp. III)	Ileum	67.8	77.4	79.9	63.5	57.2	62.3	79.8	42.2	48.1	72.1	66.1	82.3	66.9	75.4	70.4	77.1	68.9±1.
	Feces	72.0	87.1	81.6	66.1	67.4	71.2	82.9	75.1	72.3	66.5	68.7	68.7	65.7	73.6	67.6	72.3	73.2±2.
RSM-1[5] (Exp. II)	Ileum	64.2	77.5	79.1	66.8	63.0	66.9	81.0	56.6	59.7	72.1	69.1	80.1	75.7	76.0	66.4	74.0	70.4±2.
	Feces	69.2	80.3	82.2	68.7	70.1	74.2	84.9	78.5	73.0	66.5	69.5	59.9	70.8	75.1	64.7	72.9	74.3±2.
B.napus[6] Bronowski (Exp. III)	Ileum	63.3	79.0	81.4	60.6	56.5	64.1	76.8	59.9	59.9	70.4	69.7	81.0	73.7	74.1	68.1	73.3	72.3±5.
	Feces	72.8	87.8	86.2	70.9	74.8	77.3	86.4	79.1	77.3	72.3	75.5	76.8	75.2	78.4	72.7	77.9	77.9±3.

[1]Data from Tables 23 and 38.

[2]Isolated soybean protein (Nutritional Biochemicals Co., Cleveland, Ohio).

[3]B. campestris meal, prepress-solvent, Agra Vegetable Oil, Nipawin.

[4]B. campestris meal, prepress-solvent, Western Canada Seed Processors, Lethbridge.

[5]B. napus, low erucic var., solvent, Saskatchewan Wheat Pool, Saskatoon.

[6]B. napus cv. Bronowski, solvent, Saskatchewan Wheat Pool, Saskatoon.

Experiment II.

There also was a reasonably close agreement in amino acid digestibilities, whether based on feces or digesta from the ileum, for the rapeseed meals in Experiment II (RSM-1 and RSM-5) and Experiment III (regular B. napus and regular B. campestris meal). Amino acid digestibility coefficients on the basis of digesta from the ileum were similar for the regular Bronowski meal (Exp.III) and RSM-1 (Exp.II), a low-erucic acid B. napus meal. Amino acid digestibility coefficients for B. campestris meal (Exp.III) were consistently lower than those for RSM-5 (Exp.II), a B. campestris meal. On the other hand, digestibility coefficients on the basis of fecal analysis were similar for the regular B. campestris meal and RSM-5 whereas amino acid digestibilities were higher for the regular Bronowski meal than for RSM-1. Furthermore the results of the Experiment III indicated that the amino acid digestibility coefficients for the regular B. campestris and B. napus Bronowski rapeseed meals improved appreciably between the ileum and feces particularly for histidine, arginine, aspartic acid, threonine, and serine, whereas in Experiment II there was very little improvement in digestibilities for these amino acids with either rapeseed meal. The apparent discrepancies in amino acid digestibilities of rapeseed meal probably related to the fact that, although the meals used in Experiment II and III were from the same species, they differed in regard to variety, year of production, thioglucoside content, etc...

It appears that changes in digestibility between the ileum and feces depended not only on the particular amino acid under consideration but the type of protein involved. For example, amino acid digestibility coefficients were considerably higher for feces than ileum for amino acids

such as lysine, aspartic acid, threonine, serine, proline and glycine whereas little difference occurred with alanine, methionine, leucine, tyrosine and phenylalanine. The improvement in digestibility for the first group of amino acids also was more pronounced with soybean isolate than with rapeseed protein. One reason for the greater improvement with soybean may be related to the relatively high solubility of the residual nitrogen in digesta from the ileum for soybean isolate compared to rapeseed meal (Tables 18 and 33). The higher proportion of soluble nitrogen may favor fermentation in the large intestine. Amino acid digestibilities based on fecal analysis appear to give a reasonably good estimate for amino acids such as histidine, arginine, alanine, isoleucine, tyrosine and phenylalanine whereas there appears to be overestimation of the availability of amino acids such as lysine, aspartic acid, threonine, serine and glutamic acid. On the other hand methionine digestibility appears to be markedly underestimated particularly when this amino acid is limiting in the diet as in the case of soybean protein. The present data suggest that differences in amino acid digestibilities between the ileum and feces are the result of microbial rearrangement of the amino acids. Thus, availabilities based on fecal analysis may lead to erroneous conclusions for certain amino acids as has been suggested by Payne et al. (1968) and Cho and Bayley (1972).

4.3.5 Nitrogen utilization with pigs fed soybean and rapeseed protein

Table 41 summarizes nitrogen retention and feed intake for pigs fed high and low fiber soybean and rapeseed proteins. Nitrogen retention (gm of nitrogen retained/5 days) was significantly ($P<0.05$) higher for pigs fed the Bronowski RSM and soybean meal than for pigs fed the isolated soybean protein. In general, nitrogen retention appeared to be related to differences in feed intake as evidenced by the high correlation ($r = 0.89$) between feed intake and nitrogen retention. However, covariance analysis indicated that nitrogen retention was significantly ($P<0.01$) affected by factors other than feed intake which correspond to the significant differences in nitrogen retention among treatments when expressed as percent of nitrogen intake. Although apparent protein digestibility of rapeseed meal was significantly ($P<0.05$) lower than that of soybean protein (Table 29) nitrogen retention either expressed as % of nitrogen intake or as % of nitrogen absorbed, was significantly ($P<0.05$) higher with the rapeseed meal samples than with soybean protein. More efficient utilization of the protein in RSM was particularly evident when nitrogen retention was expressed as percentage of nitrogen absorbed. There were no significant differences in nitrogen utilization among the three sources of RSM.

Fiber had no consistent effect on nitrogen retention and feed intake. However feed intake was significantly ($P<0.05$) higher for pigs fed soybean meal than the soybean isolate. Feed intake also was significantly ($P<0.05$) higher for pigs fed soybean meal than for pigs fed rapeseed meal except for those fed the Bronowski meal. As in the case of Experiment I, feed intake was highest with the Bronowski rapeseed meal, a low thioglucoside meal and lowest with the B. napus, a high

Table 41. Percent of nitrogen retention by growing pigs fed regular and low fiber rapeseed meals, soybean meal and soybean isolate.

Items	Protein Source											
				Rapeseed								
	Soybean			B. campestris			B. napus			B. napus cv. Bronowski		
	Meal[1]		Isolate[2]	Meal		Low Fiber	Meal		Low Fiber	Meal		Low Fiber
Nitrogen Retention (gm N retained/ 5 days)	32.0[3] ± 6.7	(24.7)[4]	17.4 ± 7.2	23.0 ± 8.4	(23.9)	24.7 ±11.3	26.4 ± 5.5	(26.6)	24.7 ± 6.6	36.4 ± 5.3	(32.4)	28.4 ± 8.3
	ab[5]	B[6]	c	bc	B	bc	abc	AB	bc	a	A	abc
Nitrogen Retention (as % of N intake)	43.1 ± 7.0	(39.6)	36.1 ± 6.2	44.2 ± 6.5	(45.7)	47.2 ± 7.9	51.1 ± 6.6	(50.8)	50.4 ± 3.8	49.3 ± 5.2	(48.2)	47.0 ± 6.4
	ab	B	b	ab	A	a	a	A	a	a	A	a
Nitrogen Retention (as % of N absorbed)	51.1 ± 9.5	(47.2)	43.3 ± 7.7	60.7 ± 7.9	(61.1)	61.5 ±12.4	68.0 ± 6.0	(66.0)	64.0 ± 4.2	63.8 ±13.0	(61.5)	59.1 ± 6.5
	bc	B	c	ab	A	ab	a	A	ab	ab	A	ab
Feed Intake (gm)	2776 ± 232	(2340)	1904 ± 498	1954 ± 601	(1947)	1939 ± 737	1838 ± 657	(1854)	1869 ± 429	2642 ± 478	(2423)	2204 ± 374
	a	AB	bc	bc	AB	bc	c	C	c	ab	A	abc

[1]44% protein.

[2]Isolated soybean protein (Nutritional Biochemicals Co., Cleveland, Ohio).

[3]All values are means ± $S.D._{\bar{x}}$ for 5 pigs.

[4]Mean value for the two adjacent groups.

[5]Values in the same row followed by the same small letter did not differ significantly ($P<0.05$).

[6]Values in the same row followed by the same capital letter did not differ significantly ($P<0.05$).

thioglucoside rapeseed meal.

Thus the limiting factor in the utilization of rapeseed meal appears to be the overall lower feed intake generally obtained with the high thioglucoside variety of RSM rather than impaired metabolism with these diets. In fact the present results suggest that RSM protein is utilized more efficiently than soybean protein even though the digestibility of the latter protein is higher than that of RSM.

4.4 In vitro pepsin and pancreatin digestion of rapeseed meal and other protein sources.

4.4.1 Pepsin digestion

Changes in soluble nitrogen, sulfosalicylic-soluble (SA-Sol) nitrogen and free amino nitrogen during *in vitro* pepsin digestion are summarized in Table 42. The same information is illustrated in Figure 2. Percent soluble protein in 0.1 N hydrochloric acid (pH 1.5) varied from 3.6 for zein to 44.8 for soybean isolate. There was a marked improvement in the solubility of the proteins as a result of *in vitro* pepsin digestion. The solubility of soybean isolate increased to 91.4% after only one-half hour of digestion. Similarly solubility of the other protein sources increased from 37.2 to 69.7% for casein, 40.5 to 64.0% for rapeseed meal, 23.0 to 57.1% for autoclaved casein and 3.6 to 16.8% for zein during the first half hour of *in vitro* digestion. As digestion time increased all proteins tended to become highly soluble (86.1 to 99.5%) although the improvement in solubility was more rapid during the first few hours of digestion. The solubility of zein which was only 3.6% in 0.1 N HCl, tended to increase more slowly than that of the other protein sources during *in vitro* pepsin digestion but by 16 hours there were essentially no differences in solubility among the various protein sources.

Table 42. Changes in soluble nitrogen, sulfosalicylic acid soluble nitrogen (SA-Sol N) and amino nitrogen during *in vitro* pepsin digestion of various protein sources.

Source of Nitrogen	Protein Source	Blanks[1]		Length of Incubation (Hr) with Pepsin						
		0	32	½	1	2	4	8	16	32
Soluble Nitrogen (% of Total)	Casein	37.2	65.4	69.6	79.3	82.7	83.7	85.4	92.0	96.9
	Autoclaved Casein	23.0	48.0	57.1	67.6	71.1	75.6	77.4	86.5	92.2
	Soybean Isolate	44.8	76.5	91.2	93.3	95.0	96.7	98.2	98.8	99.5
	Zein	3.6	0.2	16.8	23.2	43.5	65.4	76.5	89.2	92.3
	Rapeseed Meal	40.5	45.1	64.0	67.0	72.8	77.7	82.3	84.4	86.1
	Soybean Meal	34.6	41.7	56.3	61.5	67.5	74.9	79.9	88.5	95.8
Sulfosalicylic Acid-Soluble Nitrogen (% of total)	Casein	0.5	0.5	64.8	71.7	78.9	82.2	83.8	86.3	89.0
	Autoclaved Casein	8.7	13.1	48.4	54.1	60.8	64.8	70.0	74.3	75.9
	Soybean Isolate	4.6	4.6	78.5	80.0	81.7	82.8	85.8	87.8	89.1
	Zein	0	0	16.5	22.7	43.1	61.5	74.8	88.6	92.0
	Rapeseed Meal	12.1	11.9	35.5	45.9	47.4	50.6	53.1	56.8	60.2
	Soybean Meal	3.5	4.4	55.6	57.1	66.0	67.5	68.7	74.1	75.1
Amino Nitrogen (% of total)	Casein	4.2	4.8	4.8	6.0	6.7	7.0	7.3	7.8	8.4
	Autoclaved Casein	4.4	4.6	5.3	6.0	6.8	7.2	7.4	7.6	8.2
	Soybean Isolate	3.9	3.3	5.0	5.3	6.0	6.1	6.1	7.3	8.7
	Zein	0.6	0.7	1.6	2.3	2.6	3.3	3.4	4.4	5.2
	Rapeseed Meal	2.1	1.3	3.3	4.3	5.0	5.6	6.1	6.3	6.6
	Soybean Meal	1.8	2.6	3.7	4.6	6.1	6.4	6.6	7.1	8.1

[1]Incubated under the conditions described for the protein sources but without pepsin addition.

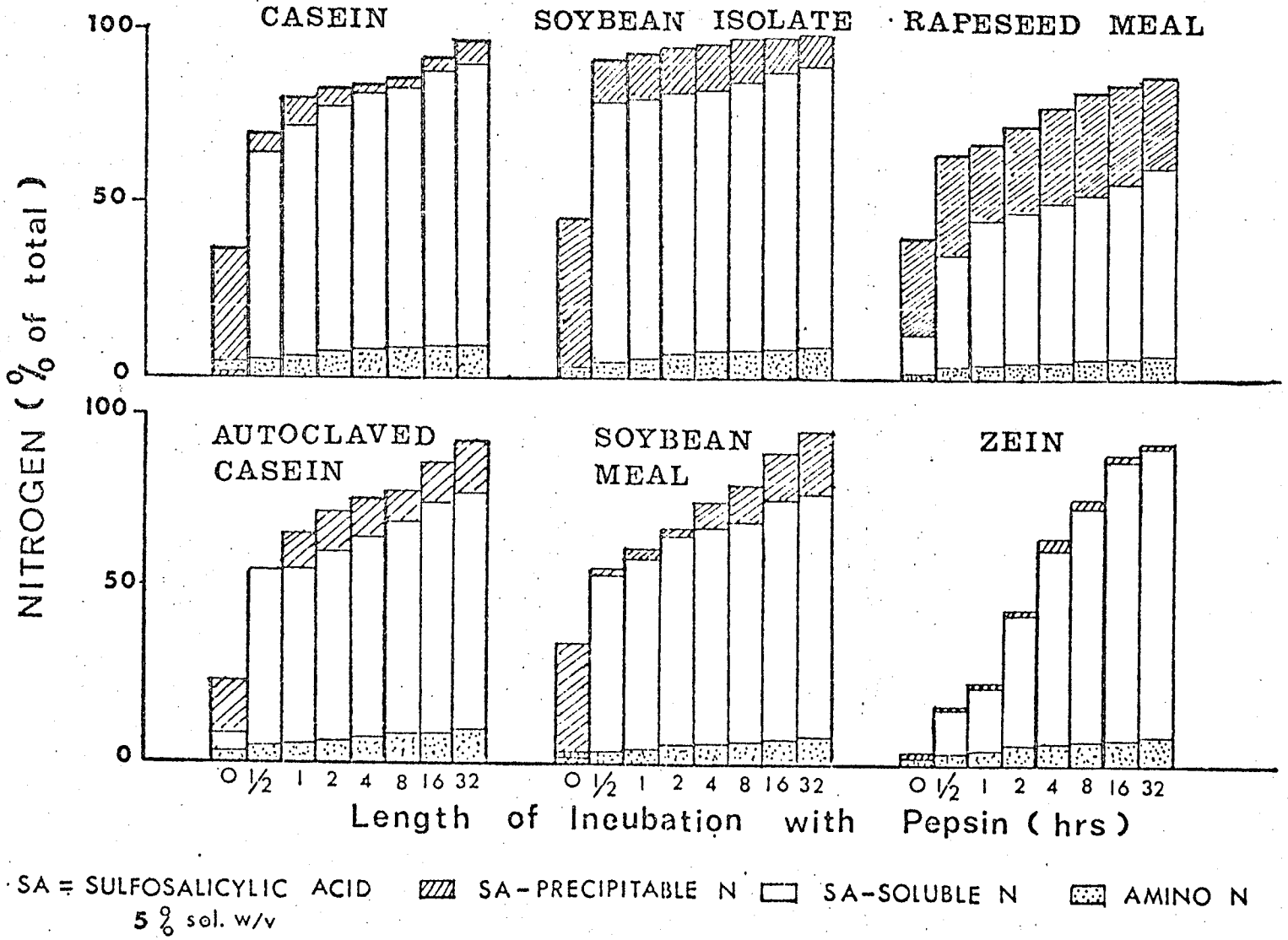

Figure 2. Changes in soluble nitrogen, sulfosalicylic acid soluble nitrogen and amino nitrogen during in vitro pepsin digestion of various protein sources.

Degree of digestion by pepsin as determined by formol titratable amino groups bore little relationship to the solubility of the various protein sources. In general there was only a 4-5% increase in free amino nitrogen irrespective of protein source during 32 hours of *in vitro* pepsin digestion. Any apparent difference in free amino nitrogen content among protein sources was due to variations in the initial free-amino nitrogen content of the proteins. However size of the individual fragments released by *in vitro* pepsin digestion as estimated by the amount of soluble nitrogen in 5% sulfosalicylic acid solution differed appreciably among protein sources. Although the total amount of SA-Sol nitrogen after ½ hour of incubation was highest for soybean isolate (78.5% of total N) and lowest with zein (16.5%), the proportion of soluble N precipitated by SA was much higher with rapeseed meal than for the other protein sources. In fact the proportion of precipitable protein ranged from 35 to 40% with rapeseed meal as compared to 10-15% with autoclaved casein and soybean isolate and only 1-7% with zein and casein. There was a very small proportion of precipitable protein (1-5%) with soybean meal during the early stages of *in vitro* digestion but the proportion of precipitable protein increased to 10-20% of the soluble N as digestion progressed and solubility increased. The proportion of soluble nitrogen precipitated by SA varied appreciably among protein sources but the amount of precipitable nitrogen remained constant within each protein source irrespective of the solubility of the protein. The only exception to this general rule was for soybean meal. Furthermore, although there were no appreciable differences among proteins in the amount of free amino nitrogen released as the result of *in vitro* pepsin digestion, there was considerable variation in solubility patterns among protein sources.

These results suggest that the length of peptide chains produced as a result of pepsin digestion varied among protein sources even though there was no difference among proteins in free amino nitrogen released during digestion. Solubility of the various proteins did not appear to be a factor in the degree of digestion of protein nor was there any indication of a direct relationship between degree of splitting and the proportion of SA-Sol nitrogen. It is possible that the solubility of zein depended on the degree of pepsin digestion because the increase in solubility of zein was linear during the first 4 hours.

4.4.2 Pancreatin digestion

Results on *in vitro* pancreatin digestion are presented in Table 43 and Figure 3. Solubility of the various proteins in phosphate buffer (0.2 M, pH 8.0) varied appreciably. Soybean isolate and casein were highly soluble (97.4 and 91.0%, respectively) compared to soybean meal (55.6%), autoclaved casein (42.9%) and rapeseed meal (38.4%). Zein which was poorly soluble in 0.1 N HCl acid (Figure 2) also was poorly soluble (3.3%) in phosphate buffer. There was an appreciable increase in solubility of autoclaved casein and rapeseed meal during the first half hour of pancreatin digestion. The solubility of autoclaved casein increased from 42.9 to 76.0% while the solubility of rapeseed meal increased from 38.4 to 70.7%. By contrast solubility of soybean meal increased from 55.6 to 66.7% and zein increased from 3.3 to 16.9%. As in the case of pepsin digestion all proteins tended to become completely soluble as incubation time was increased. The solubility ranged from 88 to 96% for all proteins, except zein after 4 hours of *in vitro* pancreatin digestion.

There was no appreciable difference in the overall rate of digestion of the various proteins estimated by the release of formol

Table 43. Changes in soluble nitrogen, sulfosalicylic acid soluble nitrogen (SA-SOL N) and amino nitrogen during *in vitro* pancreatin digestion of various protein sources.

Source of Nitrogen	Protein Source	Blanks[1]		Length of Incubation (Hr) with Pancreatin						
		0	32	½	1	2	4	8	16	32
Soluble Nitrogen (% of Total)	Casein	91.0	97.2	91.8	98.4	96.0	96.2	97.5	97.7	97.8
	Autoclaved Casein	42.9	52.6	76.0	89.0	91.6	95.6	95.9	94.4	95.8
	Soybean Isolate	97.4	96.3	95.5	96.6	96.9	95.9	99.0	97.7	98.4
	Zein	3.3	6.4	16.9	28.0	41.0	57.4	73.9	92.4	93.5
	Rapeseed Meal	38.4	44.7	70.7	76.7	83.2	88.8	92.2	92.7	95.3
	Soybean Meal	55.6	65.4	66.7	73.2	85.8	89.1	88.5	92.2	94.8
Sulfosalicylic Acid-Soluble Nitrogen (% of Total)	Casein	1.5	1.9	81.7	87.5	91.1	93.3	92.9	93.7	96.3
	Autoclaved Casein	14.6	19.4	64.6	78.2	83.2	84.4	86.8	86.5	87.0
	Soybean Isolate	1.4	1.6	72.4	78.7	81.2	86.4	87.5	86.9	90.5
	Zein	2.2	2.1	15.7	25.9	34.3	53.0	68.7	85.6	88.8
	Rapeseed Meal	15.3	21.3	38.6	46.4	56.2	66.8	68.7	69.7	72.6
	Soybean Meal	9.2	9.7	20.5	24.9	30.5	38.3	42.9	50.2	52.0
Amino Nitrogen (% of Total)	Casein	5.4	6.3	8.2	9.4	10.7	11.4	13.2	16.2	17.6
	Autoclaved Casein	3.9	3.9	5.8	6.2	7.5	8.7	9.6	11.2	13.6
	Soybean Isolate	3.2	5.8	4.7	5.6	7.2	9.0	11.1	14.2	17.9
	Zein	0.1	0	0.5	0.7	1.7	3.7	5.2	9.9	15.0
	Rapeseed Meal	1.8	3.1	2.9	3.4	4.7	6.6	8.1	10.9	15.3
	Soybean Meal	2.9	4.1	3.8	4.2	5.1	6.8	8.4	11.6	13.8

[1]Incubated under the conditions described for the protein sources but without pancreatin addition.

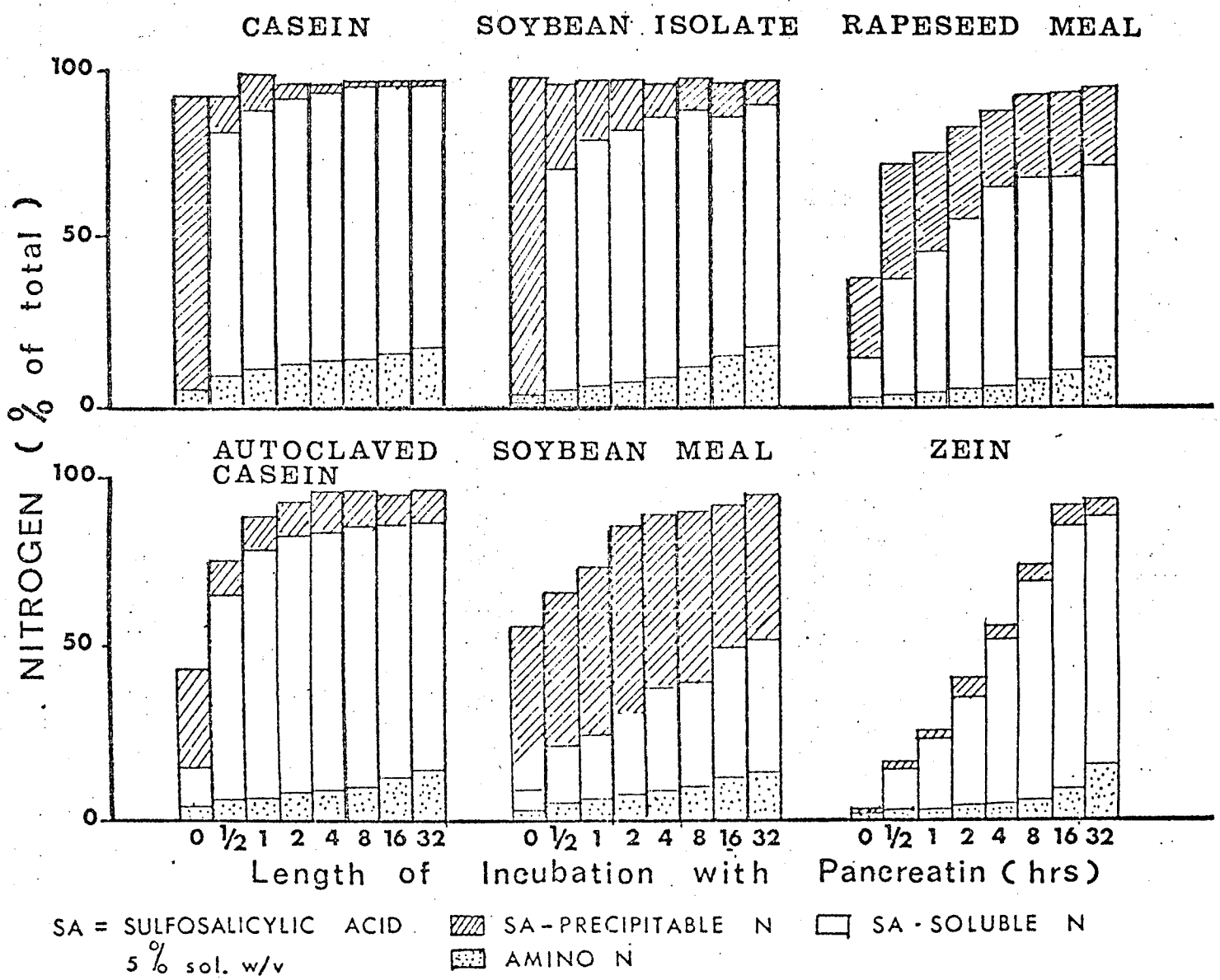

Figure 3. Changes in soluble nitrogen, sulfosalicylic acid soluble nitrogen and amino nitrogen during in vitro pancreatin digestion of various protein sources.

titratable amino groups. However rate of digestion during the first hour of incubation (Table 43) tended to be higher for casein and lower for zein than for the other protein sources. In general, the release of amino nitrogen by pancreatin digestion was two or three times that of *in vitro* pepsin digestion. The increase in amino nitrogen following 32 hours incubation with pancreatin ranged from 12-14% compared to only 4-5% with pepsin. As in the case of digestion by pepsin there was little relationship between the increase in SA-Sol nitrogen and the release of free amino nitrogen. Furthermore there was little relationship between solubility of protein and the proportion of sulfosalicylic acid soluble nitrogen. During the first half hour of incubation with pancreatin SA-Sol nitrogen increased from 1.5 to 81.7% with casein, 1.4 to 72.4% with soybean isolate, 14.6 to 64.6% with autoclaved casein, 15.3 to 38.6% with rapeseed meal, 2.2 to 15.7% with zein and 9.2 to 20.5% with soybean meal (Table 43). There was also considerable variation among proteins in the proportion of protein precipitated by sulfosalicylic acid. The proportion of precipitable protein in the soluble nitrogen fraction was markedly higher with soybean meal and rapeseed meal than with the other protein sources. Precipitable protein expressed as a percent of soluble N varied from 50 to 75% with soybean meal and from 25 to 45% with rapeseed meal as compared to only 1 to 7% with casein and zein, 8 to 12% with autoclaved casein and 8 to 23% with soybean isolate. However the proportion of precipitable protein when expressed as a percent of the total nitrogen remained relatively constant throughout the digestion period for autoclaved casein, soybean meal, rapeseed meal and zein. A similar phenomenon was also observed for all protein sources, except soybean meal, during pepsin digestion. It is possible that this phenomenon is merely the result of an equilibrium between the

concentration of substrate and digestion products. This postulate is supported by the gradual slowdown in the production of SA-Sol nitrogen and the amount of free amino nitrogen released during digestion.

The results of these in vitro studies suggest that extent of digestion of proteins by pancreatin depended upon the tertiary structure of protein rather than their solubility per se. For example, it appeared that the initial rate of digestion was more rapid with casein than soybean isolate although both proteins were nearly completely soluble in the phosphate buffer. Autoclaving appeared to alter the pattern of pancreatin attack on casein. In general, release of free amino nitrogen was less and the size of protein fragments released was greater, as indicated by the rather high proportion of SA-precipitable nitrogen, for autoclaved casein than for casein. It is possible that the specific secondary and tertiary structure of rapeseed meal protein may be responsible for the incomplete degree of digestion of this protein. Unfortunately little information is available on the relationship between protein structure and in vitro digestion of proteins.

4.4.3 Pepsin-pancreatin digestion

Changes in soluble nitrogen, sulfosalicylic acid soluble (SA-Sol) nitrogen and free amino nitrogen as the result of in vitro pepsin-pancreatin digestion (pepsin, 4 hours followed by pancreatin, 32 hours) are illustrated in Figure 4. In general, pattern of solubility for the various proteins during pepsin digestion was similar to that observed with pepsin in the preceding experiment. As in the preceding experiment the solubility of nitrogen after 4 hours of pepsin digestion was markedly higher with soybean isolate, casein and autoclaved casein than with rapeseed meal, soybean meal and zein. However total soluble nitrogen

Table 44. Changes in soluble nitrogen, sulfosalicylic acid soluble nitrogen (SA-Sol N) and amino nitrogen during *in vitro* pepsin-pancreatin digestion of various protein sources.

Source of Nitrogen	Protein Source	Length of Incubation (Hr) with Enzymes											
		Pepsin				Pancreatin							Blank[1]
		0	1	2	4	0	1	2	4	8	16	32	32
Soluble Nitrogen (% of Total)	Casein	43.4	71.7	72.7	80.3	94.1	99.7	98.7	99.0	98.0	98.4	98.3	98.5
	Autoclaved Casein	29.2	63.3	68.5	80.8	82.1	87.9	94.0	97.1	96.7	97.3	95.3	56.0
	Soy Isolate	40.8	79.1	80.6	89.2	99.3	99.3	99.8	96.5	98.5	94.9	97.6	97.9
	Zein	3.5	13.4	32.1	57.1	55.7	70.8	83.5	84.5	87.6	91.8	96.2	6.2
	Rapeseed Meal	36.1	42.4	52.1	57.9	57.7	90.6	92.2	94.2	95.6	93.6	96.2	47.6
	Soybean Meal	35.2	49.6	52.1	54.8	62.0	80.0	81.0	88.0	87.9	90.1	92.9	65.9
Sulfosalicylic Acid-Soluble Nitrogen (% of Total)	Casein	1.9	58.6	69.0	75.7	77.1	96.6	94.5	94.2	93.4	96.7	97.6	1.6
	Autoclaved Casein	6.5	45.6	49.9	54.7	50.8	85.2	90.6	89.4	91.9	93.9	91.8	13.8
	Soy Isolate	2.5	68.0	69.5	78.1	79.5	89.2	90.1	89.2	92.3	90.1	94.7	1.2
	Zein	0.2	11.5	31.9	54.7	53.8	68.2	74.6	81.4	82.4	87.7	92.5	0.4
	Rapeseed Meal	16.6	35.0	39.8	43.7	42.0	59.6	68.6	76.3	81.4	84.0	84.0	18.3
	Soybean Meal	3.0	44.3	48.9	49.3	49.2	55.8	64.1	73.3	75.6	77.3	84.1	2.7
Amino Nitrogen (% of Total)	Casein	4.1	5.2	5.4	6.0	6.3	11.3	13.1	14.0	15.6	18.3	18.6	5.7
	Autoclaved Casein	3.3	4.9	5.9	6.1	6.4	8.9	10.2	11.6	13.9	15.0	18.8	4.3
	Soy Isolate	2.6	3.8	3.9	4.2	4.3	8.6	9.1	10.7	12.3	16.5	18.3	3.2
	Zein	0.2	1.6	2.2	3.1	3.6	5.0	6.1	8.2	10.7	13.5	14.8	0.3
	Rapeseed Meal	2.5	3.7	4.7	5.4	5.2	7.6	8.6	9.3	11.3	13.4	15.9	3.8
	Soybean Meal	2.4	3.6	4.0	5.6	5.3	8.3	10.4	12.2	14.2	14.8	15.2	3.1

[1]Incubated under the conditions described for the protein sources but without pepsin and pancreatin addition.

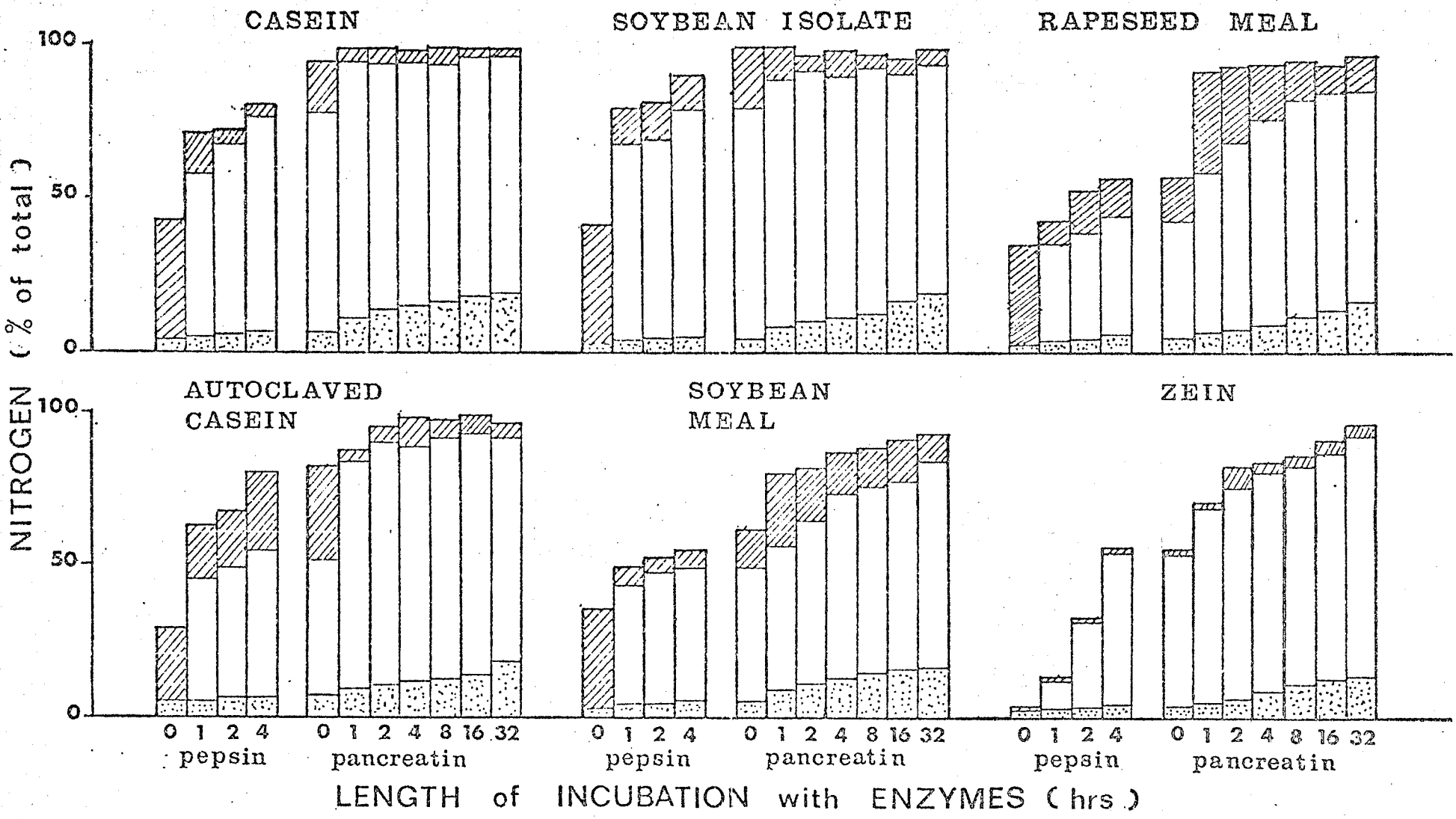

Figure 4. Changes in soluble nitrogen, sulfosalicylic acid soluble nitrogen and amino nitrogen during in vitro pepsin-pancreatin digestion of various protein sources.

tended to be lower in the present study which is probably related to the smaller amount of pepsin used (15 mg/gm of protein) than in the earlier experiment (40 mg/gm of protein).

Digestion with pancreatin resulted in a rapid increase in the solubility of all proteins, except casein and soybean isolate which were highly soluble in phosphate buffer (Figure 4, 0 hours, pancreatin digestion). Improvement in solubility was particularly pronounced with rapeseed meal which increased from 57.9 to 90.6% during the first half hour of digestion. In fact after two hours of pancreatin digestion, solubility of all protein sources, except zein and soybean meal was greater than 92%.

Degree of digestion estimated by the amount of formol titratable amino groups was markedly improved as a result of preliminary pepsin digestion (amino nitrogen data, Table 43 vs. Table 44). Release of amino nitrogen was consistently higher for similar periods of incubation with pancreatin when the proteins had been previously subjected to pepsin digestion. The effect of preliminary pepsin digestion was particularly evident during the first hour of pancreatin digestion. As digestion continued the amount of amino nitrogen released tended to be similar for the two digestion systems. Release of amino nitrogen during the two first two hours of digestion was higher for casein than for the other protein sources with both the pancreatin and pepsin-pancreatin systems. These results coincide with the concept that pepsin is not required for the in vitro digestion of casein.

The amount of SA-Sol nitrogen also was markedly improved by preliminary pepsin digest (Table 44 vs. Table 43). One of the main differences between the two digestion systems was the marked reduction in the percentage of precipitable protein for soybean meal and rapeseed

meal when incubation with pepsin preceded pancreatin digestion. Precipitable protein constituted a constant 45% of the total nitrogen in the incubation mixture with soybean meal and 30% with rapeseed meal when incubated with pancreatin alone. By contrast SA-precipitable protein gradually decreased from 22% after 1 hour of pancreatin digestion to 9% after 32 hours with soybean meal and from 33% to 9% with rapeseed meal when these proteins were pre-digested with pepsin. Similarly pre-digestion of autoclaved casein and soybean isolate with pepsin resulted in a slightly but consistently lower percent of precipitable protein during pancreatin digestion. On the other hand pre-digestion had no effect on the proportion of precipitable protein with casein and zein where the proportion of SA precipitable protein was negligible with pancreatin digestion alone.

The present study suggests that pre-digestion by pepsin is of particular importance to the rate and extent of pancreatin digestion for certain proteins. These results agree with those of Sheffner et al. (1956) who found that pre-digestion with pepsin reduced the variation in the degree of digestion of proteins by trypsin. The present results also suggest that protein structure may be an important factor in protein digestion since a considerable improvement occurred in the in vitro pancreatin digestion of rapeseed meal and soybean meal as a result of preliminary digestion with pepsin. Size of the peptides released by pepsin may be an important factor in the extent of digestion of rapeseed protein by pancreatin. Rapeseed meal was the only protein for which there was a considerable amount of SA-precipitable protein even after 32 hours of pepsin digestion which suggests that pepsin is only partially effective in splitting rapeseed protein into small fragments.

4.4.4 Release of free amino acids during pepsin-pancreatin digestion.

Amino acid composition of nitrogen containing fragments that were soluble in sulfosalicylic acid and the levels of free amino acids in this fraction were determined for each of the protein sources in an attempt to further define the extent and pattern of digestion during the early stages of pepsin-pancreatin digestion (Table 45). Amino acid composition of the SA-Sol fraction did not differ appreciably from that of the corresponding dietary proteins. In general, amino acid composition of the SA-Sol fraction and dietary protein agreed within ±10% which is the maximum deviation usually accepted in our laboratory for analysis on duplicate hydrolysates of the same samples. The similarity of pattern suggests that no particular fraction(s) were more soluble in SA as the result of digestion by pepsin and pancreatin. Although the pattern of amino acids in the SA soluble fraction coincided with that in the diet, the actual amount of each amino acid in the SA-Sol fraction was lower with zein, rapeseed meal and soybean meal than with casein, autoclaved casein and soybean isolate because of the lower proportion of SA-Sol nitrogen with zein, rapeseed meal and soybean meal.

Release of free amino acids during pepsin/pancreatin digestion was determined by amino acid analyses of the non-hydrolyzed SA-fraction and the results were expressed as the percent of each amino acid present in the intact protein or as percent of the amino acid present in the hydrolyzed SA-Sol fraction (Table 46). Free amino acids released during incubation were mainly those specifically liberated by pancreatin. The extent of release usually was in the following order: arginine > phenylalanine > tyrosine > lysine > leucine > methionine. These amino acids accounted for 50 to 70% of the total amino nitrogen released. Release of the other amino acids was generally negligible. The proportion of the

Table 45. Amino acid composition of the test proteins and the sulfosalicylic acid soluble nitrogen (SA-Sol N) fraction, and amount of free amino acid released during a one-hour *in vitro* pepsin-pancreatin digestion.

Source of Nitrogen	Protein Source	Amino Acids																
		Lys	His	Arg	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val	Met	Ile	Leu	Tyr	Phe
Test Proteins[1] (a.a.,gm/16 gm N)	Casein	7.74	2.65	3.53	8.13	5.15	6.09	25.02	11.65	2.10	3.45	-	7.12	3.22	5.47	11.11	6.68	5.91
	Autoclaved Casein	7.65	2.76	3.70	8.00	4.64	5.35	24.61	12.35	2.14	3.57	-	7.08	2.94	5.34	10.70	6.84	6.13
	Soybean Isolate	5.73	2.26	8.06	12.75	3.96	5.33	20.51	5.54	4.30	4.17	-	4.62	1.35	4.78	9.24	4.39	6.23
	Zein	0.24	1.25	1.56	5.80	2.95	5.81	25.23	9.45	1.28	10.01	0.20	3.60	1.09	3.79	21.61	4.34	7.22
	Rapeseed Meal	5.06	2.49	5.91	7.65	4.67	4.81	19.63	7.11	5.50	4.62	-	5.09	1.85	3.87	7.57	3.02	3.89
	Soybean Meal	6.40	2.42	7.75	13.02	4.35	5.28	20.87	6.19	5.05	5.16	0.986	4.87	1.31	4.59	8.16	4.34	5.25
SA-Sol N[1] (a.a., gm/16 gm N in SA fraction)	Casein	8.48	2.82	3.89	8.08	4.83	5.91	24.11	11.38	2.25	3.46	-	6.70	3.04	5.39	10.62	6.18	5.51
	Autoclaved Casein	7.28	2.57	3.63	9.31	5.09	5.79	24.78	12.85	2.34	3.48	-	6.57	2.70	5.10	10.47	6.28	5.71
	Soybean Isolate	5.80	2.60	7.91	12.88	4.22	5.38	20.78	5.89	4.49	4.23	-	4.78	1.18	4.68	8.64	4.44	6.14
	Zein	0.35	1.42	2.25	6.30	3.73	5.77	23.64	11.10	1.50	9.99	-	3.48	1.43	4.19	20.93	5.15	7.61
	Rapeseed Meal	5.52	2.77	6.46	8.85	4.72	4.32	18.51	6.90	6.08	4.76	0.53	4.74	2.26	3.75	6.89	3.66	4.48
	Soybean Meal	6.91	2.46	8.39	12.99	4.40	5.26	20.61	5.44	5.12	4.79	0.73	4.48	1.26	4.34	7.02	4.15	4.79
SA-Sol N[1] (Gm. of a.a. soluble in SA per 100 gm of incubated test protein)	Casein	7.84	2.61	3.62	7.50	4.48	5.49	22.38	10.57	2.09	3.22	-	6.22	2.82	5.01	9.86	5.74	5.12
	Autoclaved Casein	6.16	2.17	3.07	7.87	4.31	4.90	20.97	10.87	1.98	2.94	-	5.56	2.28	4.32	8.86	5.31	4.83
	Soybean Isolate	5.14	2.30	7.00	11.40	3.73	4.76	18.39	5.21	3.97	3.74	-	4.23	1.05	4.14	7.65	3.93	5.44
	Zein	0.23	0.95	1.51	4.22	2.50	3.87	15.84	7.44	1.00	6.69	-	2.33	0.96	2.81	14.03	3.45	5.10
	Rapeseed Meal	3.06	1.54	3.58	4.91	2.61	2.39	10.26	3.83	3.37	2.64	0.29	2.63	1.25	2.08	3.82	2.03	2.48
	Soybean Meal	3.63	1.40	4.41	6.82	2.31	2.76	10.83	2.86	2.69	2.52	0.38	2.35	0.66	2.28	3.69	2.18	2.52
Free Amino Acids[2] (gm/100 gm of incubated test protein)	Casein	5.49	0.34	3.58	0.20	0.22	0.27	0.83	0.07	0.14	0.29	-	0.29	1.27	0.14	5.70	4.58	4.46
	Autoclaved Casein	3.68	0.11	1.71	0.05	0.10	0.17	0.40	0.06	0.10	0.29	-	0.20	0.47	0.11	2.39	1.96	2.14
	Soybean Isolate	2.17	0.21	5.74	0.11	0.11	0.25	0.24	-	0.07	0.15	-	0.23	0.30	0.41	3.13	2.16	2.96
	Zein	0.22	0.12	1.48	0.33	0.25	0.55	1.60	0.72	0.14	1.53	-	0.31	0.38	0.32	5.66	2.02	3.57
	Rapeseed Meal	1.66	0.25	3.23	0.19	0.36	0.35	0.99	0.30	0.12	0.26	1.01	0.81	0.34	0.42	2.38	1.56	1.96
	Soybean Meal	1.76	0.15	3.91	0.30	0.17	0.45	0.72	0.08	0.11	0.33	-	0.43	0.21	0.48	2.24	1.42	2.33

[1]The test proteins and the SA-Sol N (sulfosalicylic acid soluble nitrogen) fraction were hydrolyzed with 6 N HCl for 22 hrs at 105°C.

[2]Free amino acid content was determined directly by ion exchange chromatography on the SA-Sol N fraction after dilution of sample with pH 2.2 sodium citrate buffer.

Table 46. Percent of release[1] of individual amino acids during a one-hour *in vitro* pepsin-pancreatin digestion of rapeseed meal and other protein sources.

Amino Acids	Amino Acids Released during Digestion (%)[2]						Amino Acids Released in Sulfosalicylic Acid Soluble Nitrogen Fraction (%)[3]					
	Casein	Autoclaved Casein	Soybean Isolate	Zein	Rapeseed Meal	Soybean Meal	Casein	Autoclaved Casein	Soybean Isolate	Zein	Rapeseed Meal	Soybean Meal
Arginine	101.4	46.2	71.3	94.5	54.7	51.4	98.9	55.6	82.0	97.9	90.2	88.7
Phenylalanine	75.5	36.2	47.5	49.5	50.5	44.4	87.2	22.1	54.4	70.1	79.0	92.5
Tyrsoine	68.6	28.7	49.3	46.6	51.5	32.8	79.9	36.9	55.1	58.7	76.8	65.3
Lysine	71.0	48.1	37.8	93.6	32.7	27.5	70.1	59.8	42.2	94.3	54.1	48.5
Leucine	51.8	22.3	33.9	26.2	31.4	27.4	57.8	27.0	41.0	40.4	62.5	60.6
Methionine	39.6	15.7	22.4	35.0	18.5	16.2	45.2	20.2	28.9	39.8	27.3	31.7
Alanine	8.3	8.0	3.7	15.2	5.7	6.3	8.9	9.7	4.1	22.8	10.0	12.9
Isoleucine	2.6	2.1	8.5	8.5	10.8	10.8	2.8	2.6	9.8	11.4	20.0	20.9
Valine	4.1	2.8	5.0	8.7	15.9	8.9	4.7	3.6	5.4	13.5	30.9	18.4
Histidine	12.9	4.1	9.2	9.8	9.9	6.3	13.1	5.3	9.0	12.9	16.0	10.9
Threonine	4.3	2.2	2.7	8.6	7.7	4.0	5.0	2.3	2.8	10.1	13.7	7.5
Serine	4.4	3.1	4.6	9.4	7.2	8.6	4.9	3.4	5.2	14.1	14.5	16.4
Glycine	6.7	4.8	1.6	10.7	2.2	2.1	6.7	5.1	1.7	13.6	3.5	4.0
Glutamic Acid	3.3	1.6	1.2	6.3	5.1	3.4	3.7	1.9	1.3	10.1	9.7	6.6
Aspartic Acid	2.4	0.6	0.9	5.7	2.4	2.3	2.6	0.6	1.0	7.9	3.8	4.4
Proline	0.6	0.5	0	7.6	4.1	1.3	0.7	0.5	-	9.7	7.7	2.9
% Free Amino Acids[4] (including ammonia)	36.3	18.8	26.9	29.4	26.8	22.2	38.6	22.1	29.4	41.9	43.0	39.9

[1] Calculated from the data presented in Table 45.

[2] $\frac{\text{Free amino acid (gm) released}}{\text{Amino acid (gm/16 gm N) content of test proteins}} \times 100$

[3] $\frac{\text{Free amino acid (gm) released}}{\text{Amino acid (gm/100 gm of incubated test protein}} \times 100$

[4] $\frac{\text{Summation of free amino acid released in gm.}}{\text{Total protein (100 gm) in incubation mixture or SA-Sol N fraction}} \times 100$

amino acids listed above that were released by pancreatin varied among protein sources; release generally being higher for casein and lower for autoclaved casein than for the other protein sources. The greater release of free amino acids with casein as the result of pepsin-pancreatin digestion coincided with the fact that there was a greater amount of formol titratable amino groups with casein than with the other proteins, particularly in the early stages of pepsin-pancreatin digestion. One of the interesting features in the in vitro release of amino acids was the high proportion of arginine released with all protein sources except autoclaved casein. In addition one hour incubation of zein with pancreatin resulted in an almost complete liberation of arginine and lysine and a relatively high release of methionine compared to the release of this amino acid with the other protein sources except casein. Whether the nearly complete release of lysine and arginine as free amino acids with zein is related to their low content in this protein or their particular position in the molecule is not resolved in the present experiment. On the other hand the percent of lysine released during incubation was markedly lower with soybean isolate, soybean meal and rapeseed meal than with casein, autoclaved casein and zein. However when free amino acid content was expressed as percent of the SA-Sol nitrogen there was no appreciable difference among casein, rapeseed meal and soybean meal for arginine, tyrosine, phenylalanine and leucine, whereas the percentage of free lysine and methionine was still lower with rapeseed meal and soybean meal. Arginine was primarily present in free form except in the case of autoclaved casein.

The present results tend to agree with those of Hankes et al. (1948) and Riesen et al. (1947) who found that autoclaving reduced the in vitro pepsin/pancreatin release of all amino acids. However the

present results differ from those of Evans and Butts (1949), Ford (1965) and Ford and Salter (1966) who found that autoclaving had a more pronounced effect on the in vitro trypsin/erepsin release of lysine than other amino acids. It is possible that the poor release of lysine, as in the case with autoclaved casein may in turn affect the release of other amino acids by pancreatin. On the other hand the similarity of pattern in the amino acid composition of the SA-Sol nitrogen fraction and that of dietary proteins suggests no differential release of amino acids during the initial splitting of the protein molecule. However the amount of free amino acid released during digestion varied both with amino acids and protein sources. Thus the accessibility of the individual amino acids to hydrolytic release by pepsin and pancreatin may be of importance to the rapidity and extent of digestion of the proteins. For example the accessibility of the peptide bond adjacent to lysine in rapeseed meal, soybean meal and soybean isolate might be responsible for the rather poor release of this amino acid compared to arginine during in vitro incubation. Similarly the particular position of arginine in the peptide chain might explain the relatively high release of this amino acid with all protein sources except autoclaved casein. Results of the present study suggest that the rate and extent of digestion of protein, estimated either by the amount of SA-Sol nitrogen or by the release of formol titratable amino groups, give an inaccurate picture of the digestion of the protein. For example a considerable portion (50 to 70%) of the amino acids in the SA-Sol nitrogen fraction was present as peptides even though more than 85% of protein in casein, autoclaved casein and soybean isolate was soluble in sulfo-salicylic acid. One of the limitations in the determination of free amino groups by formol titration is the fact that it gives no indication

of the amino acids contributing the amino groups or whether these amino acids are in free or peptide form. The lower amount of formol titratable amino groups with zein compared to the other protein sources was probably related to the low amount of lysine and arginine in this protein even though release of these amino acids was nearly complete. Furthermore the relatively poor release of amino acids other than arginine, lysine, tyrosine, phenylalanine and leucine during this pepsin-pancreatin digestion may reflect more the specificity of the proteolytic enzymes used during *in vitro* digestion than the actual susceptibility of the amino acids to enzymatic hydrolysis per se. Thus any conclusion on the *in vitro* release of amino acids from any particular protein source must be interpreted with caution.

Chapter V

DISCUSSION

The primary object of the present study was to investigate the reason(s) for the consistently lower digestibility of protein in RSM compared to that of other protein sources (Drouliscos and Bowland, 1968; Oliver et al., 1970; Cho and Bayley, 1970; May and Bell, 1971). Experiment I investigated the effect of method of processing (expeller, prepress-solvent and solvent processes) on nitrogen utilization of RSM by growing pigs. Twelve samples of RSM prepared from seeds harvested in 1967 and 1968 were received from 5 different processors. Eight of the samples were prepared from the cultivar B. campestris, three from common B. napus varieties and one from B. napus var. Bronowski. Neither method of processing nor variety of RSM had any influence on the digestibility of dry matter, protein and individual amino acids in RSM. Drouliscos and Bowland (1968) and Saben et al. (1971a) also found no effect of processing or variety on dry matter and protein digestibility of rapeseed meal. Methods of processing also had no effect on nitrogen retention (Table 9). However, variety of RSM had a marked effect on feed intake and nitrogen retention (gm N/pig/4 days). Nitrogen retention was higher ($P < 0.05$) for pigs fed Bronowski meal than for those fed the regular B. napus meals but did not differ ($P < 0.05$) from those fed the B. campestris meals. Analysis of covariance and expression of nitrogen retention as percent of N intake or percent of N absorbed indicated that differences in nitrogen retention among varieties was primarily due to differences in feed intake. A high correlation between feed intake and nitrogen retention also was found by Bell (1957), Belzile et al. (1963) and Bell et al. (1971) with mice fed various samples of RSM.

The present results suggest that thioglucoside level in the various rapeseed meals did not interfere with nitrogen utilization per se although thioglucoside level in RSM appeared to interfere with feed intake.

In Experiment II, protein digestibility by the growing pig was determined for casein, autoclaved casein, soybean isolate, zein and three samples of rapeseed meal. Apparent and true digestibility of the RSM samples was significantly ($P<0.05$) lower than that of casein and soybean isolate. Protein digestibility for the rapeseed meal samples also tended to be lower than that of autoclaved casein and zein although the difference was significant ($P<0.05$) only in the case of one rapeseed meal sample (RSM-5). These observations support earlier reports that protein digestibility of rapeseed meal is lower than that of soybean meal (Droulis©os and Bowland, 1968; Cho and Bayley, 1970; May and Bell, 1971), casein (Droulis©os and Bowland, 1968; Oliver et al., 1970), barley, wheat and fishmeal (May and Bell, 1971).

Samples of digesta were collected from various regions of the alimentary tract in an attempt to explain the lower digestibility of rapeseed protein. Although apparent protein digestibility of casein was consistently higher than that of RSM in all regions of the intestinal tract, digestibility of casein based on digesta from the ileum was significantly ($P<0.05$) higher than only one sample of rapeseed meal (RSM-1). Protein digestibility of zein in the ileum tended to be lower than that of the other protein sources including the three rapeseed meal samples although the values with zein were highly variable. An interesting observation with respect to protein digestibility was the marked improvement ($P<0.05$) in the digestibility of soybean isolate and zein between the ileum and feces whereas little improvement in digestibility occured with casein, autoclaved casein and the rapeseed meals. The improvement

in digestibility between the ileum and feces for soybean isolate and zein was reflected in a two-fold decrease in the protein/Cr_2O_3 ratio for these proteins (Table 47) compared to little or no changes with casein, autoclaved casein and the rapeseed meal samples. Cho and Bayley (1972) also observed a two-fold decrease in the protein/PEG and amino acids/PEG ratios between the ileum and rectum of pigs fed soybean meal. They interpreted the greater concentration of PEG in the rectal samples as an indication of the extent to which food residues are fermented and fermentation products absorbed. Unfortunately these researchers were unable to determine similar relationships with rapeseed meal because of the unsatisfactoriness of PEG as a digestibility index with this protein. Results of the present study (Table 47) also suggest that fermentation occurred with zein and soybean isolate during transit through the large intestine. Fermentation may have occurred with soybean isolate and zein because of the amount and the specific nature of the non-digested protein entering the large intestine. The relatively low amount of protein in digesta of pigs fed casein and the protein-free diet probably limited the extent of fermentation of residual protein in the caecum and colon. Michel (1966) reported that catabolic activity in the caecum was more pronounced when digesta contained a high proportion of non-digested material as in the case of impaired absorption or diarrhea. In addition gastrectomy in the human and the accompanying accumulation of large amounts of non-digested residues has been reported to result in considerable fermentation in the ileum and large intestine (Lundh, 1958). Less fermentation may have occurred with autoclaved casein than with soybean and zein because of the possible effect of the Maillard reaction on microbial breakdown of the protein. The apparent lack of fermentation with RSM may have been due to the relatively high proportion of fibrous

Table 47. Protein to chromic oxide (Cr_2O_3) ratios in the diet, digesta and feces of pigs fed rapeseed meal and other protein sources[1].

Source of Sample	Protein Source							
					Rapeseed Meal			
	Casein	Autoclaved Casein	Soybean Isolate	Zein	RSM-1	RSM-4	RSM-5	Protein Free
Diet	62.7	62.8	62.0	62.1	62.6	64.1	62.8	-
Stomach	68.4[2] ±33.3	44.4 ± 5.5	59.4[2] ±20.6	50.2 ± 9.5	53.2 ±10.9	55.9 ± 7.9	54.0 ± 6.8	6.8 ± 1.2
Mid jejunum	18.1 ± 3.3	35.0 ± 9.3	29.6 ± 1.1	46.6[2] ±11.3	39.6 ±14.4	25.7 ± 2.4	30.3 ± 8.4	13.4 ± 2.3
Ileum	8.7[2] ± 2.9	13.8[2] ± 5.4	18.6[2] ± 2.0	24.6[2] ±10.4	18.6[2] ± 1.5	17.3[2] ± 3.3	14.4[2] ± 2.8	6.8[2] ± 1.3
Caecum	7.3 ± 1.5	14.6 ± 0.6	15.9 ± 1.7	11.1 ± 2.1	16.8 ± 1.2	17.8 ± 2.7	16.8 ± 0.9	5.4 ± 0.6
Feces[3]	5.3 ± 0.8	13.2 ± 1.6	9.9 ± 0.7	12.2 ± 2.9	15.6 ± 1.9	14.5 ± 1.4	16.0 ± 1.1	4.5 ± 0.3

[1]All values in digesta and feces are mean ± $S.D._x$ for 4 pigs except as indicated by superscript[2].

[2]Mean ± $S.D._x$ for 3 pigs.

[3]Five-day fecal collection.

material in the digesta of pigs fed this protein. Fiber may have affected fermentation either by direct inhibition or through acceleration of intestinal transit.

Results of Experiment III indicated that the lower digestibility of protein for rapeseed meal than for soybean meal was associated, in part, with the hull fraction of the meal. Removal of the hull fraction from rapeseed meal (B. campestris, B. napus and B. napus var. Bronowski meals) resulted in an overall improvement in protein digestibility. In fact, protein digestibility of the low-fiber Bronowski meal was equivalent ($P<0.05$) to that of soybean meal or soybean isolate although digestibility of rapeseed protein was, in general, lower ($P<0.05$) than that of soybean protein. Fiber had no effect on the digestibility of soybean protein.

The fact that removal of the hull fraction from rapeseed meal resulted in a marked improvement in protein digestibility coincides with the observation of Bayley et al. (1969) that steam pelleting and regrinding RSM resulted in an increase in crude fiber digestibility and a concomitant improvement in dry matter, nitrogen and energy digestibility. These observations also substantiate the results by various researchers (Hussar and Bowland, 1959b; Manns et al., 1963b; Cho and Bayley, 1970; Saben et al., 1971; Bowland and Schuld, 1968; Bayley et al., 1969; Bowland, 1971) that the digestibility of protein in RSM generally follows that of dry matter and energy.

Protein digestibility coefficients based on digesta from the rectum and caecum also were consistently ($P<0.05$) lower for the rapeseed samples than for soybean meal or soybean isolate. However no differences were found among protein sources when digestibility coefficients were

based on digesta from the ileum and the mid jejunum. As in Experiment II, there was a significant ($P<0.05$) improvement in protein digestibility for soybean isolate between the ileum and feces but only a slight improvement with the regular rapeseed meals. Improvement also occurred with soybean meal in Experiment III. Similarly, an appreciable improvement in digestibility was observed between the ileum and feces for the low-fiber rapeseed meals although improvement was statistically significant ($P<0.05$) only in the case of the low-fiber B. napus and low-fiber Bronowski meals. Improvement in digestibility for the low-fiber rapeseed meals between the ileum and feces suggests that fiber associated with the hull fraction reduced fermentation in the large intestine. The crude fiber content of the various meals was 13.0 -13.3% with the regular rapeseed meals, 6.5 - 8.2% with the low-fiber rapeseed meals and 5.3% with soybean meal.

In general digestibility of individual amino acids followed that of total protein for each of the dietary treatments. Digestibility coefficients of amino acids from casein were significantly ($P<0.05$) higher than those of the other protein sources which coincides with the results of Carlson and Bayley (1970) and Flipot et al., 1971. Autoclaving significantly ($P<0.05$) reduced the digestibility of all amino acids in casein. Amino acid digestibility coefficients for rapeseed meals were usually lower than those of the other protein sources. Removal of the hull fraction from rapeseed meal, however, resulted in an improvement in amino acid digestibility although the difference was significant ($P<0.05$) only in the case of the B. campestris meal. In general digestibility coefficients of amino acids in the various protein sources in the present study agreed fairly closely with those reported for the same protein sources (Carlson and Bayley, 1970; Cho and Bayley,

1970; Tao et al., 1971; Flipot et al., 1971; Sauer, 1972).

There was no consistent pattern, however, in amino acid digestibility coefficients within the various protein sources. Differences in apparent digestibility among amino acids appeared a function of the levels of the amino acids in the diet and the levels of amino acids in metabolic nitrogen. For example, the lower apparent digestibility of methionine with soybean meal, soybean isolate and zein compared to casein, autoclaved casein and the rapeseed meals corresponded to the lower content of methionine in soybean and zein. Similarly, the relatively low digestibility of glycine with all proteins, except rapeseed meal, coincided with the relatively low level of this amino acid in these diets. Metabolic amino acids had an appreciable effect on digestibility of individual amino acids as evidenced by the fact that true digestibility coefficients within each protein source were much less variable than apparent digestibility coefficients. However, there was no consistent pattern in true amino acid digestibility coefficients although with the exception of casein, true digestibility of glutamic acid, histidine, and arginine tended to be higher than that of the other amino acids, particularly glycine, alanine, threonine, and methionine. Similar patterns of amino acid digestibility have been observed for a variety of proteins (Olsen, 1967; Olsen et al., 1968; Neilson, 1968; Cho and Bayley, 1970; Giovanetti et al., 1970; Carlson and Bayley, 1970; Sauer, 1972). No completely satisfactory explanation can be offered at present for either the differences in digestibility among amino acids or the lower overall digestibility of amino acids in rapeseed meal.

The validity of amino acid digestibility coefficients might be seriously questioned in view of the fact that fecal amino acid patterns

tended to be similar for the various dietary treatments including the protein-free diet (Tables 12, 20, 35). Nasset (1957) and Gouwens (1966) also observed uniform fecal amino acid patterns with widely different dietary patterns. They suggested that the similarity in fecal amino acid patterns was the result of either endogenous protein secretion or microbial synthesis. However endogenous amino acids did not completely mask the effect of the diet. In general amino acid composition of digesta and feces in Experiment II and III reflected major differences in composition among the various dietary proteins. Influence of dietary amino acids was particularly evident in the case of zein wherein the relatively high content of leucine, alanine and phenylalanine and the low content of lysine, histidine and arginine in the diet was reflected by a similar pattern in digesta and feces. The relatively high content of aspartic acid in soybean protein also was reflected in the digesta and feces in both Experiment II and III. These observations support the general conclusion (Crompton and Nesheim, 1969; Carlson and Bayley, 1970; Nixon and Mawer, 1970a) that dilution of digesta and feces by endogenous protein is insufficient to mask the amino acid pattern of the diet. On the other hand amino acid composition of digesta from pigs fed casein corresponded closely to that of pigs fed the protein-free diet. These observations coincide with the general conclusion that casein was rapidly absorbed in the proximal small intestine. Thus the nitrogen present at various levels of the intestinal tract of pigs fed casein would have been mainly of endogenous origin. In fact endogenous nitrogen represented 74% or more of the total nitrogen in feces and digesta from various regions of the alimentary tract of pigs fed casein (Table 48) but only 25 to 50% of the total nitrogen in the digesta and feces of pigs fed the other protein sources. Bergen and Purser (1968)

Table 48. Contribution of the metabolic nitrogen to the total nitrogen in the digesta and feces of pigs fed rapeseed meal and other protein sources[1].

Source of Digesta	Protein Source						
					Rapeseed Meal		
	Casein	Autoclaved Casein	Soybean Isolate	Zein	RSM-1	RSM-4	RSM-5
Stomach	9.9	15.3	11.4	13.5	12.8	12.2	12.6
Mid jejunum	74.0	38.3	45.3	28.8	33.8	52.1	44.2
Ileum	78.2	49.3	36.6	27.6	36.6	39.3	47.2
Caecum	74.0	37.0	34.0	48.6	32.1	30.3	32.1
Feces[2]	84.9	34.1	45.5	36.9	28.8	31.0	28.1

[1]Contribution of the metabolic nitrogen to the total nitrogen was calculated from the data presented in Table 47 according to the following formula:

$$\% \text{ Met. N in digesta or feces} = \frac{\text{Ratio Protein/}Cr_2O_3 \text{ in digesta or feces with the protein-free diet} \times 100}{\text{Ratio Protein/}Cr_2O_3 \text{ in digesta or feces with protein diets}}$$

[2]Five-day fecal collection.

found that endogenous N accounted for over 90% of the total nitrogen in the ileum of rats fed casein compared to 66% with those fed bacterial or protozoal protein.

The present study does not support the observations by Nasset and Ju (1961) who found that overall dilution of exogenous protein with endogenous protein in the small intestine was ninefold with dogs and sevenfold with rats. It can be calculated from the data summarized in Table 47 that metabolic nitrogen in the mid jejunum of pigs fed the protein-free diet was equivalent to about 22% of the nitrogen intake when the diet contained approximately 16% protein. Part of the discrepancies between the present results and those of Nasset and Ju (1961) could be accounted for by the method used in the determination of endogenous protein. Nasset and Ju (1961) did not account for the amount of protein that had been absorbed prior to collecting samples of digesta from the small intestine. They simply calculated the proportion of endogenous to exogenous nitrogen in the small intestine of dogs and rats at various time intervals after feeding ^{14}C-labelled casein. However when secretion of endogenous N was estimated by feeding a low-protein or protein-free diet containing a non-digestible indicator (Twombly and Meyer, 1961; Crompton and Nesheim, 1969; Nixon and Mawer, 1970a) protein secretion was considerably less than that reported by Nasset and Ju (1961). Twombly and Meyer (1961) found that secretion of endogenous nitrogen into the gastrointestinal tract of rats was equivalent to the amount of nitrogen consumed when fed a 10% protein diet. Endogenous protein secretion into the small intestine of the duck was equivalent to 50% of the exogenous protein intake (Crompton and Nesheim, 1969) while in human subjects endogenous protein accounted for 13 to 53% of the protein contained in the test meals (Nixon and Mawer, 1970a).

The proportion of metabolic nitrogen at various sites along the intestinal tract was relatively constant for each protein source (Table 48) which suggests that rate of digestion of endogenous protein was about the same as that of non-digested residue between the mid jejunum and caecum. These results do not coincide with the general belief that endogenous protein is absorbed primarily in the ileum (Twombly and Meyer, 1961; Gitler, 1964; Ochoa-Solano and Gitler, 1968). However it is possible that the non-digested residues from exogenous protein were particularly resistant to digestion in the present study and were absorbed at the same rate as endogenous protein.

Marked changes were observed in the amino acid pattern of digesta between the ileum and feces of pigs fed the various diets. In general, the proportion of glycine, proline and glutamic acid which was fairly high in the ileum decreased appreciably while the proportion of alanine, methionine, isoleucine, leucine, tyrosine and phenylalanine increased. Cho and Bayley (1972) also observed a significant ($P<0.05$) increase in the proportion of isoleucine, methionine and alanine between the ileum and rectum of pigs fed soybean meal or rapeseed meal and a decrease in the proportion of proline, glycine (+ leucine) and glutamic acid. However, Cho and Bayley (1972) did not find a significant change in the proportion of tyrosine and phenylalanine. Apparent digestibility of glutamic acid, proline, glycine, aspartic acid, threonine and serine, increased between the ileum and caecum in Experiment II (Table 23) and ileum and feces in Experiment III (Table 38). Changes in amino acid digestibility pattern between the ileum and feces suggest either extensive absorption of certain amino acids in the ileum or fermentation of amino acids in the large intestine. The possibility of extensive absorption of amino acids in the ileum is indicated

by the fact that most of the changes in the amino acid composition of digesta were observed between the ileum and caecum. The decrease in the proportion of glycine between the ileum and caecum could have been due to reabsorption of bile in the ileum (Nixon and Mawer, 1970a). In addition, it is possible that the high dipeptidase activity in the mucosa of the ileum (Robinson and Shaw, 1960) might be responsible for extensive hydrolysis of specific peptides and absorption of their constituent amino acids in the ileum. In fact, Nixon and Mawer (1970b) found that the release of glutamic acid, aspartic acid and glycine was more rapid during in vitro incubation of digesta from the distal small intestine of young men than during incubation of digesta from the proximal small intestine. However there is also some question as to whether reabsorption of endogenous protein was responsible for the marked changes in amino acid pattern between the ileum and feces. Reabsorption of mucoproteins which are particularly low in lysine, methionine, leucine, tyrosine and phenylalanine and relatively rich in threonine, proline and alanine (Werner, 1953; Clarke et al., 1966) would result in a higher proportion of lysine, methionine, leucine, tyrosine and phenylalanine and a lower proportion of threonine, proline and alanine in the digesta from the caecum. In the present study, however, the proportion of alanine in digesta increased rather than decreased between the ileum and feces while threonine and lysine remained constant. Nevertheless the proportion of methionine, leucine, tyrosine and phenylalanine increased and the proportion of proline decreased between the ileum and caecum in Experiment II and ileum and feces in Experiment III.

It is now well established (Lundh, 1958; Larson and Hill, 1960; Michel, 1966; Salter and Coates, 1971) that there is considerable

bacterial activity and consequent breakdown of amino acids in the ileum, caecum and colon. It appears more plausible that the changes in the amino acid composition of digesta between the ileum and feces were the result of bacterial fermentation rather than extensive reabsorption of amino acids in the ileum. As indicated previously, feces and digesta from the caecum were characterized by a lower proportion of proline and glycine and a higher proportion of alanine, methionine, leucine, tyrosine and phenylalanine than digesta from the ileum. These findings coincide with the observations by Combe and Pion (1966) that the ethanol and TCA insoluble nitrogen from normal rats contained a lower proportion of proline and glycine and a higher proportion of alanine and phenylalanine than that from axenic rats. Microflora could have been associated with similar changes observed in amino acid pattern between the ileum and feces in the present study. The decrease in amino acids to chromic oxide ratios between the ileum and feces for all dietary treatments except autoclaved casein and rapeseed meals in Experiment II (Tables 49 and 50) is further evidence of fermentation in the large intestine. Significant reduction in the amount of amino acids and an increase in the apparent digestible nitrogen between the ileum and feces also has been reported by Kay (1969a) Cho and Bayley (1972) and Coelho Da Silva et al. (1972). Lower solubility of the nitrogen in feces and digesta from the caecum than in digesta from the ileum also supports the postulation of microbial protein synthesis in the large intestine. Combe et al. (1966) reported that 50% of total nitrogen in the caecum of rats fed a standard diet was of bacterial origin. They found that only 42 to 52% of the N from the caecum of normal rats was soluble in ethanol and TCA solution compared to 89% for axenic rats. Furthermore of interest was the fact that the amino acid composition of feces and digesta

Table 49. Individual amino acid and protein to chromic oxide (Cr_2O_3) ratios[1] in diets, digesta and feces of pigs fed the various protein sources in Experiment II.

Dietary Regimen	Source of Nitrogen	Amino Acids																
		Lys	His	Arg	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe	Protein
Casein	Diet	4.80	1.68	2.35	4.74	2.83	3.81	14.75	7.05	1.32	2.12	4.02	1.78	3.08	6.35	2.99	3.32	62.68
	Mid jejunum	1.12	0.29	0.56	1.45	0.83	0.92	2.74	2.07	2.78	0.61	0.66	0.19	0.55	0.81	0.35	0.39	16.80
	Ileum	0.54	0.11	0.27	0.68	0.57	0.51	1.70	0.66	1.46	0.38	0.40	0.12	0.27	0.43	0.23	0.21	8.59
	Caecum	0.49	0.12	0.31	0.80	0.40	0.34	0.97	0.35	0.41	0.51	0.41	0.19	0.31	0.51	0.27	0.42	7.77
	Feces	0.36	0.08	0.22	0.56	0.28	0.24	0.66	0.29	0.27	0.32	0.29	0.13	0.23	0.39	0.21	0.22	5.27
Autoclaved casein	Diet	4.20	1.56	2.18	4.50	2.73	3.68	14.84	6.99	1.27	2.05	4.06	1.71	3.15	6.13	2.94	3.25	62.80
	Mid jejunum	2.39	0.95	1.02	3.35	1.66	1.97	8.52	4.47	3.22	1.29	1.79	0.71	1.58	2.45	1.03	1.13	35.17
	Ileum	1.17	0.27	0.31	1.99	0.76	1.05	3.55	1.56	0.96	0.48	0.67	0.40	0.71	0.77	0.22	0.25	13.63
	Caecum	1.39	0.27	0.25	1.91	0.70	0.74	3.26	1.27	0.49	0.64	0.77	0.41	0.83	0.87	0.30	0.33	14.38
	Feces	1.01	0.27	0.41	1.39	0.63	0.67	2.17	0.81	0.43	0.62	0.73	0.34	0.66	0.86	0.36	0.40	12.62
Soybean Isolate	Diet	3.64	1.64	5.22	7.71	2.13	3.24	12.40	3.36	2.52	2.54	3.56	0.71	2.56	5.12	2.26	3.34	62.00
	Mid jejunum	1.46	0.55	1.61	4.23	1.27	2.03	5.70	2.66	2.90	1.21	1.42	0.40	0.95	1.59	0.74	0.97	29.33
	Ileum	0.88	0.35	0.75	3.31	1.03	1.57	4.19	1.51	1.50	0.67	0.81	0.28	0.60	0.91	0.41	0.64	18.41
	Caecum	0.90	0.26	0.64	2.23	0.82	1.10	2.63	0.77	0.84	0.77	0.88	0.25	0.65	0.96	0.41	0.66	15.69
	Feces	0.55	0.18	0.38	1.23	0.59	0.62	2.36	0.46	0.50	0.58	0.63	0.24	0.39	0.60	0.31	0.53	12.46
Zein	Diet	0.15	0.77	0.97	3.58	1.82	3.59	15.59	5.84	0.79	6.18	2.22	0.68	2.34	13.35	2.68	4.46	62.12
	Mid jejunum	0.82	0.49	1.18	2.92	1.75	2.36	9.76	4.54	5.04	3.62	1.74	0.46	1.65	6.29	1.69	2.27	45.91
	Ileum	0.30	0.37	0.50	1.34	0.86	1.17	5.46	2.84	1.06	2.24	0.88	0.29	0.95	3.80	0.99	1.46	24.35
	Caecum	0.28	0.15	0.20	0.73	0.38	0.51	2.26	0.90	0.34	0.98	0.48	0.17	0.48	1.68	0.40	0.69	11.18
	Feces	0.38	0.13	0.24	1.20	0.56	0.71	2.62	0.78	0.44	1.16	0.59	0.26	0.57	2.06	0.51	0.69	13.36
RSM-1	Diet	3.45	1.57	3.74	4.84	2.96	3.05	12.42	4.50	3.48	2.92	3.22	0.97	2.44	4.78	1.53	2.46	62.64
	Mid jejunum	2.00	0.93	1.97	3.33	2.01	2.08	6.91	4.14	4.93	1.91	1.98	0.34	1.38	2.56	0.86	1.28	44.10
	Ileum	1.24	0.35	0.78	1.61	1.10	1.01	2.36	1.95	1.40	0.81	0.99	0.19	0.59	1.15	0.51	0.64	18.54
	Caecum	1.14	0.33	0.72	1.62	0.96	0.84	2.05	1.18	0.97	1.05	0.96	0.30	0.73	1.25	0.55	0.70	16.91
	Feces	1.06	0.31	0.67	1.51	0.89	0.79	1.86	0.98	0.94	0.98	0.98	0.39	0.71	1.19	0.54	0.67	16.10
RSM-5	Diet	3.30	1.69	4.07	4.94	3.03	3.03	12.03	4.44	3.49	3.22	3.28	1.29	2.30	4.79	1.91	2.56	62.75
	Mid jejunum	1.83	0.66	1.37	2.62	1.58	1.43	4.46	2.78	3.04	1.36	1.39	0.46	0.97	1.96	0.73	1.03	30.06
	Ileum	0.91	0.26	0.48	1.29	0.86	0.73	1.73	2.23	1.14	0.65	0.73	0.17	0.52	0.96	0.36	0.52	14.31
	Caecum	1.17	0.28	0.60	1.68	0.98	0.89	2.08	1.24	1.02	0.98	1.01	0.32	0.77	1.35	0.55	0.80	16.63
	Feces	1.01	0.27	0.70	1.56	0.88	0.77	1.83	0.94	0.90	0.95	0.88	0.37	0.69	1.21	0.48	0.65	16.19
Protein-free[3]	Mid jejunum	0.35	0.14	0.53	0.69	0.47	0.51	0.82	3.49	2.22	0.51	0.42	0.09	0.20	0.46	0.21	0.23	13.44
	Ileum	0.18	0.10	0.24	0.44	0.35	0.32	0.55	1.92	1.14	0.26	0.22	0.04	0.13	0.27	0.12	0.14	6.78
	Caecum	0.34	0.09	0.23	0.54	0.30	0.26	0.64	0.56	0.42	0.32	0.28	0.13	0.21	0.38	0.16	0.21	5.44
	Feces	0.31	0.08	0.19	0.47	0.27	0.24	0.54	0.16	0.23	0.31	0.26	0.12	0.21	0.34	0.15	0.18	4.48

[1]Amino acid and protein to Cr_2O_3 ratios were computed from data in Table 23 according to the following formula:

$$\text{Ratio Protein/}Cr_2O_3\text{ in digesta and feces} = \frac{100 - \text{Protein or amino acid digestibility (\%)}}{100} \times \frac{\text{Protein or amino acid (gm) in diet}}{\text{Chromic oxide (gm) in diet}}$$

[2]The protein or amino acid to chromic oxide ratios with pigs fed the protein free diet is an average value of individual protein or amino acid to Cr_2O_3 ratios in digesta and feces.

Table 50. Individual amino acid and protein to chromic oxide (Cr_2O_3) ratios[1] in diets, digesta and feces of pigs fed regular and low-fiber rapeseed meals, soybean meal and soybean isolate (Experiment III).

Dietary Regimen	Sample	Amino Acids																
		Lys	His	Arg	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe	Protein
Soybean - Meal	Diet	3.74	1.46	4.50	7.99	2.72	3.66	13.38	3.56	2.91	2.92	2.98	1.05	2.90	5.40	2.22	3.32	66.48
	Mid jejunum	1.73	0.65	1.62	3.82	1.31	1.64	5.69	3.25	2.73	1.45	1.32	0.33	1.13	1.95	0.89	1.19	31.71
	Ileum	1.34	0.40	0.78	2.49	0.90	0.97	4.29	2.81	1.78	0.80	0.65	0.17	0.48	0.99	0.43	0.57	19.48
	Feces	0.76	0.17	0.43	1.21	0.60	0.55	1.51	0.51	0.65	0.86	0.64	0.32	0.53	0.96	0.44	0.54	11.04
- Isolate	Diet	3.54	1.45	4.84	8.08	2.45	3.37	13.30	3.15	2.66	2.40	2.87	0.79	2.91	5.67	2.38	3.43	62.00
	Mid jejunum	1.39	0.55	1.27	4.16	1.23	1.80	5.61	3.74	3.01	1.02	0.93	0.24	0.84	1.38	0.60	0.87	28.52
	Ileum	1.19	0.41	0.90	3.80	1.09	1.68	4.76	1.82	1.53	0.66	0.57	0.17	0.60	0.96	0.45	0.63	21.02
	Feces	0.59	0.18	0.41	1.29	0.53	0.68	1.52	0.46	0.52	0.54	0.47	0.25	0.44	0.73	0.32	0.47	10.04
B. campestris - Meal	Diet	3.93	1.81	3.98	5.01	3.03	3.06	13.06	4.74	3.67	3.28	3.15	1.47	2.23	5.11	1.87	2.89	65.48
	Mid jejunum	1.73	0.52	1.23	2.71	1.58	1.61	4.66	3.24	3.02	1.39	1.46	0.32	0.97	1.88	0.70	0.98	28.03
	Ileum	1.27	0.41	0.80	1.83	1.30	1.15	2.64	2.74	1.90	0.92	1.07	0.26	0.74	1.26	0.55	0.66	20.63
	Feces	1.10	0.23	0.73	1.70	0.99	0.88	2.23	1.18	1.02	1.10	0.99	0.46	0.76	1.35	0.61	0.80	17.55
- Low Fiber	Diet	3.80	1.69	4.17	5.20	3.20	3.18	13.66	4.98	3.72	3.25	3.34	1.63	2.36	5.29	1.98	2.84	66.32
	Mid jejunum	1.81	0.49	1.17	2.61	1.51	1.49	4.73	3.26	4.03	1.35	1.37	0.29	0.91	1.70	0.72	0.83	29.51
	Ileum	1.24	0.31	0.63	1.55	1.16	0.95	2.47	2.87	1.54	0.75	0.90	0.20	0.59	1.02	0.47	0.55	18.24
	Feces	0.92	0.19	0.58	1.32	0.77	0.69	1.72	1.00	0.82	0.86	0.79	0.39	0.70	1.10	0.48	0.59	13.53
B. napus- Meal cv. Bronowski	Diet	3.71	2.04	4.52	5.14	3.22	3.36	13.53	4.78	3.81	3.42	3.65	1.48	2.90	5.67	1.83	2.95	70.56
	Mid jejunum	2.14	0.76	1.63	3.39	2.03	2.10	6.56	3.86	4.08	1.82	2.03	0.43	1.31	2.65	0.91	1.30	38.67
	Ileum	1.36	0.43	0.84	2.03	1.40	1.21	3.14	1.92	1.53	1.01	1.11	0.28	0.76	1.47	0.57	0.79	19.55
	Feces	1.01	0.25	0.62	1.49	0.81	0.76	1.84	1.00	0.78	0.95	0.89	0.34	0.72	1.22	1.50	0.65	14.89
- Low Fiber	Diet	3.82	1.86	4.34	4.83	2.99	3.14	13.84	4.55	3.56	3.24	3.35	1.22	2.50	5.20	1.90	2.89	67.40
	Mid jejunum	1.82	0.62	1.51	2.69	1.42	1.48	5.20	3.82	3.25	1.65	1.51	0.41	1.04	2.07	0.75	0.99	32.89
	Ileum	1.44	0.41	0.95	2.04	1.25	1.12	3.47	2.93	2.16	1.09	1.04	0.27	0.74	1.35	0.50	0.73	22.85
	Feces	0.81	0.21	0.53	1.30	0.71	0.66	1.69	0.85	0.76	0.83	0.77	0.29	0.61	1.03	0.38	0.57	12.88

[1] Amino acids and protein to Cr_2O_3 ratios were computed from data in Table 38 according to the following formula:

$$\text{Ratio Protein/}Cr_2O_3 \text{ in digesta and feces} = \frac{(100 - \text{Protein or amino acid digestibility (\%)})}{100} \times \frac{\text{Protein or amino acid (gm) in diet}}{\text{Chromic oxide (gm) in diet}}$$

from the caecum bore several similarities with that of rumen (Sharma, 1973) and other bacteria (Stokes and Gunness, 1946; Anderson et al., 1958). In fact the higher content of alanine, methionine, isoleucine, tyrosine, and phenylalanine in feces than digesta from the ileum coincides with the relatively high content of these amino acids in bacteria. Similarly the relatively low content of proline and glycine in bacteria might explain the decrease in the proportion of these amino acids in the caecum and feces. The levels of lysine, glutamic acid, threonine and serine in feces and digesta from the caecum also bore several similarities with those of bacteria.

During the writing of this section of the thesis it was brought to the author's attention that diaminopimelic acid (DAP) can be used to estimate the proportion of bacterial nitrogen to total nitrogen in digesta (Sharma, 1973). As a result, two samples of feces, one from pigs fed soybean meal and one from pigs fed rapeseed meal, were analyzed for DAP by the method of Hirs (1967). There was 0.50 g and 0.36 g DAP per 16 gm of N in the feces of pigs fed soybean meal and rapeseed meal, respectively. If one assumes that fecal bacteria contains a similar amount of DAP to that of rumen bacteria (0.89 gm/16 gm of nitrogen - Hutton et al., 1971) then 56 and 40% of the total fecal N would be of bacterial origin for pigs fed soybean meal and rapeseed meal, respectively. These findings substantiate the suggestion of Osborne and Mendel (1914) and more recently Salter and Coates (1971) that much of the non-digested protein is ultimately incorporated into bacterial cells and excreted as such. The presence of DAP would have led to an overestimation of the methionine in feces because the two are not resolved by ion exchange chromatography. Diaminopimelic acid accounted for 34.4 and 30.6% of the methionine found in the feces of pigs fed soybean meal and rapeseed meal,

respectively. These observations also explain the apparent anomaly wherein apparent and true digestibility coefficients for methionine tended to be higher in the ileum than in the feces. For example, apparent digestibility of methionine on the basis of digesta from the ileum was 84.2% for soybean meal and 82.3% for regular B. campestris rapeseed meal compared to 69.6% and 68.7% respectively on the basis of fecal analysis (Table 38). Correction of fecal methionine for DAP resulted in apparent digestibility coefficients of 80.3% for soybean meal and 78.0% for rapeseed meal which coincides with the digestibility coefficients based on ileal digesta. Failure to account for the presence of DAP in feces would result in an underestimation of the availability of methionine, particularly for proteins that are low in methionine and may explain the low digestibility of methionine in soybean meal (Cho and Bayley, 1970; Giovanetti et al., 1970; Sauer, 1972).

Evidence that an appreciable proportion of fecal N was of microbial origin raises the question whether degree of fermentation varied with the type of protein ingested and whether certain amino acids were more susceptible to fermentation. Comparison of amino acid digestibility coefficients based on ileal digesta with those based on feces indicated that there was considerable variation in the digestibilities of individual amino acids between these two sites (Tables 23, 24, 38, 39). Although digestibility coefficients for alanine, isoleucine, leucine, tyrosine and phenylalanine were similar for the ileum and feces, an appreciable improvement generally occurred between the ileum and feces for aspartic acid, threonine, serine and proline. Digestibility of methionine, on the other hand, tended to decrease from the ileum to the feces with most protein sources. Changes in amino acid digestibility between the ileum and feces in Experiment II usually were more pronounced

for soybean isolate and zein than for casein, autoclaved casein and rapeseed meals. The same pattern prevailed in Experiment III except that the difference between soybean protein and rapeseed protein was less pronounced because of the substantial improvement in amino acid digestibility with rapeseed meal between the ileum and feces. The fact that improvement in digestibility with soybean meal was consistently higher for aspartic acid, threonine, serine, glutamic acid, proline and glycine than for alanine, methionine, isoleucine, tyrosine and phenylalanine is in agreement with the results of Cho and Bayley (1972) although the magnitude of changes was more pronounced in their experiment. They reported a three fold reduction in amino acid/PEG ratios between the ileum and feces for arginine, aspartic acid, threonine, and a two fold reduction or less with valine, tyrosine, isoleucine, lysine and alanine. In the present experiment (Table 50) there was a three fold decrease for glutamic acid, proline and glycine but only a one to two fold decrease for aspartic acid, serine and threonine and no reduction in alanine, valine, isoleucine, leucine, tyrosine and phenylalanine. No decrease in the methionine/indicator ratio was found in either study. It is interesting that the amino acids for which there was a greater improvement in digestibility were those which have been found most susceptible to breakdown during incubation with microflora from the caecum of pigs (Michel, 1966). Thus it is possible that amino acid digestibility coefficients based on fecal analysis might reflect the relative susceptibility of the amino acids to microbial breakdown rather than their true availability. This phenomenon might explain why the digestibilities for proline and glutamic acid are generally higher than those of the other amino acids (Carlson and Bayley, 1970; Giovanetti et al., 1970; Sauer, 1972) even though endopeptidases such

as pepsin, trypsin and chymotrypsin do not specifically hydrolyze proteins or peptides adjacent to these amino acids (Hill, 1965). By contrast the relatively small improvement in digestibility for alanine and methionine may be related to their greater resistance to microbial breakdown and explain why the digestibility of these amino acids is relatively low compared to that of the other amino acids. Thus amino acid digestibility based on fecal analysis might lead to an overestimation of the availability of proline, glycine, aspartic acid, threonine, serine and glutamic acid. Similarly availability may also be underestimated when the proportion of amino acid in a diet is markedly lower than that of metabolic fecal nitrogen which will explain the low availability of lysine in zein (Experiment II) and cereals (Giovanetti et al., 1970; Sauer, 1972). The presence of DAP will result in an underestimation of the availability of methionine particularly in diets limiting in that amino acid. However amino acid digestibility based on fecal analysis would appear to give a satisfactory estimate of the availability of alanine, isoleucine, leucine, tyrosine and phenylalanine. Furthermore amino acid digestibility coefficients based on fecal analysis appear to give a fairly good estimate of the availability of amino acids with highly digestible proteins such as casein or proteins such as rapeseed meal and autoclaved casein which do not seem to be readily fermented in the large intestine. The present study suggests that digestibility values based on fecal analysis must be used with caution in the determination of the availability of amino acids in proteins and agrees with the suggestion of Payne et al. (1968) that amino acid digestibility based on digesta from the ileum represents a more accurate index of amino acid availability.

The fact that the nitrogen retention expressed as % of N intake

tended to be higher for rapeseed meals than soybean isolate and zein even when these proteins were supplemented with limiting amino acids (Table 27) supports the hypothesis that the difference in digestibilities among these proteins is primarily the result of fermentation. Furthermore postprandial plasma amino acid levels at one and three hours after feeding the test diets suggested similar rates of absorption for rapeseed meal and soybean isolate (Tables 25 and 26).

In vitro digestion with pepsin, pancreatin and pepsin-pancreatin bore little relationship to apparent digestibility coefficients for rapeseed meal and the other protein sources when fed to growing pigs (Table 51). Thus in vitro digestion, determined either by the release of amino acid N or amount of sulfosalicylic acid soluble nitrogen (SA-Sol N), did not satisfactorily explain differences in apparent digestibility among the protein sources used in this study. However, there were some interesting observations associated with in vitro digestions. Total soluble nitrogen and SA-Sol N were similar for zein which suggests that the relatively low apparent digestibility of this protein, based on digesta from the ileum, may be related to the low solubility of the protein prior to partial digestion. However, solubility did not appear to be an important factor in the in vitro digestion of casein, autoclaved casein, soybean isolate, rapeseed meal and soybean meal. In fact with these protein sources total soluble nitrogen was much greater than SA-Sol N during the early phases of in vitro digestion which tends to rule out the possibility that differences in digestibility were associated with solubility. Although in vivo digestion of the various protein sources did not correlate well with in vitro assays, preliminary digestion of the proteins with pepsin increased overall agreement between digestibility and in vitro digestion. In fact, in

Table 51. Comparison of *in vitro* digestion of rapeseed meal and other protein sources to that of the apparent digestibility of these proteins.

In Vitro Digestion	Length of Incubation	Source of Nitrogen	Protein Source: Casein	Autoclaved Casein	Soybean Isolate	Zein	Rapeseed Meal	Soybean Meal
Pepsin	1 hr	Sol N[2]	79.3	67.6	93.3	23.2	67.0	61.5
		SA-Sol N[3]	71.7	54.1	80.0	22.7	45.9	57.1
		Amino N[4]	6.0	6.0	5.3	2.3	4.3	4.6
	16 hr	Sol N	92.0	86.5	98.8	89.2	84.4	88.5
		SA-Sol N	86.3	74.3	87.8	88.6	56.8	74.1
		Amino N	7.8	7.6	7.3	4.4	7.1	7.1
Pancreatin	1 hr	Sol N	98.4	89.0	96.6	28.0	76.7	73.2
		SA-Sol N	87.5	78.2	78.7	25.9	41.4	24.9
		Amino N	9.4	6.2	5.6	0.7	3.4	4.2
	16 hr	Sol N	97.7	94.4	97.7	92.4	92.7	92.2
		SA-Sol N	93.7	86.5	86.9	85.9	69.7	50.0
		Amino N	16.2	11.2	14.2	9.9	10.9	11.6
Pepsin-Pancreatin	1 hr	Sol N	99.7	87.9	99.3	70.8	90.6	80.0
		SA-Sol N	96.6	85.2	89.2	68.2	59.6	55.8
		Amino N	11.3	8.9	8.6	5.0	7.6	8.3
	16 hr	Sol N	98.4	97.3	94.9	91.8	93.6	90.1
		SA-Sol N	96.7	93.9	90.1	87.7	84.0	77.3
		Amino N	18.3	15.0	16.5	13.5	13.4	14.8
In Vivo Digestion								
App.Dig. (%)		Ileum	86.3	78.3	70.3[5](68.2)[6]	60.8	70.4[7](71.8)[9]	70.8
		Feces	91.6	79.3	84.2[5](83.9)[6]	80.8	75.5[8](75.8)[9]	83.4

[1]RSM-5, a low-erucic acid B. napus meal, solvent processed.

[2]Soluble nitrogen (% of total N).

[3]Sulfosalicylic acid soluble nitrogen (% of total N).

[4]Amino N by formol titration using glycine as standard and expressed as percent of the total N.

[5]Apparent digestibility of soybean isolate in Experiment II.

[6]Average apparent digestibility of soybean isolate in Experiment II and III.

[7]Apparent digestibility of RSM-1 in Experiment II.

[8]Average apparent digestibility of all regular RSM samples in Experiment II and III.

in vitro pepsin-pancreatin digestion at one hour agreed fairly closely with apparent digestibility coefficients, based on ileal digesta, for casein, autoclaved casein and zein. However, there was a rather poor relationship between apparent digestibility and free amino acid N released during *in vitro* incubation for isolate soybean meal and between apparent digestibility and SA-Sol N for soybean isolate, rapeseed meal and soybean meal. Evans *et al.* (1947) and Sheffner *et al.* (1956) also found that predigestion with pepsin reduced the variation associated with *in vitro* assays using trypsin alone. However, there are several problems associated with *in vitro* assays including the enzyme system used, the length of incubation, the criteria used to assess degree of digestion, etc.

Release of individual amino acids during pepsin-pancreatin digestion followed a similar pattern for all proteins with the only amino acid released in significant amounts being those specific to pepsin and pancreatin. The percent of total amino acid released occurred in the following order: arginine> phenylalanine> tyrosine> lysine> leucine> methionine. These amino acids accounted for 50 to 70% of the total amino acids released. Similar observations were reported by Nixon and Mawer (1970b) when incubating the intestinal content of man fed either casein or gelatin. The nearly complete release of arginine with pepsin-pancreatin for all protein sources, except autoclaved casein, could account for the high digestibility generally observed for arginine in a variety of protein sources (Olsen *et al.*, 1968; Giovanetti *et al.*, 1970; Sauer, 1972). However, the poor release of amino acids which are not specific to pepsin and pancreatin emphasizes the difficulty of comparing digestibility of amino acids with the release of amino acids during *in vivo* digestion. Although *in vitro* digestion assays did not appear to

be satisfactory for the assessment of digestibility of different protein sources, release of free amino acids with pepsin-pancreatin digestion may be useful in comparing the effect of processing on the utilization of a single protein source (casein vs. autoclaved casein, Table 51). Fecal amino acid analysis still appears to be the most satisfactory method of estimating the availability of amino acid per se, even though the possibility of fermentation in the large intestine casts doubt on the reliability of these values, especially for some amino acids. More research needs to be carried out on the relative importance of fermentation in the large intestine particularly with respect to protein source and other constituents in the diet (e.g. fiber).

Neither the *in vivo* nor the *in vitro* studies completely explain the consistently lower digestibility of rapeseed meal protein compared to other protein sources such as casein, zein or soybean protein. Nevertheless the overall results suggest that the utilization of rapeseed protein is equivalent to that of soybean protein and seeming differences in digestibility appear to be related to the relative susceptibility of these proteins to fermentation in the large intestine. Results of the present study suggest that the primary limiting factor in the utilization of rapeseed meal is the low feed intake generally associated with the regular meals available commercially. The introduction of low thioglucoside varieties appears to be particularly promising since feed intake with Bronowski rapeseed meal was similar to that of soybean meal in Experiment III.

Chapter VI

SUMMARY AND CONCLUSIONS

Three experiments involving 6-week old castrated male piglets, were carried out in an attempt to explain the lower digestibility reported for rapeseed meal compared to casein, soybean meal and cereals.

Experiment I compared the digestibility and nitrogen utilization of 12 samples of rapeseed meal from 5 different processors. Apparent protein digestibility for the 12 RSM samples ranged from 71.0 to 77.9% while apparent digestibility of individual amino acids varied from 69.0 to 84.6%. Methods of processing (expeller, prepress-solvent or solvent), variety of rapeseed (B.campestris, regular B.napus, B.napus cv. Bronowski) and year of harvest (1967, 1968) had no effect on apparent and true digestibility of protein and amino acids. These parameters also had no effect on the efficiency of nitrogen utilization when expressed either as percent of nitrogen intake or as percent of the nitrogen absorbed. Nitrogen retention (grams N per pig/4 days) however, was significantly ($P<0.05$) higher for Bronowski meal than for the regular B.napus meals while N retention for the B.campestris meals, with one exception, tended to be intermediate between these two sources. Covariance analysis indicated that the higher nitrogen retention for Bronowski meal and one sample of B.campestris meal than for the regular B.napus meals was a function of feed intake. No general pattern was found for the digestibility of the individual amino acids, except for the consistently higher digestibility of glutamic acid, histidine and arginine.

In Experiment II protein digestibility and pattern of digestion of three samples of rapeseed meal (B.napus cv. Oro, B. napus cv. Bronowski,

B.campestris) were compared with those of casein, autoclaved casein, soybean isolate and zein. True and apparent protein digestibility coefficients were significantly ($P<0.05$) lower for the rapeseed meals than for casein and soybean isolate but did not differ appreciably from those of autoclaved casein and zein. Apparent protein digestibility coefficients for the three rapeseed meal samples were 75.3, 77.5 and 74.7 (Oro, Bronowski and B.campestris meals, respectively) compared to 91.8 for casein, 84.2 for isolated soybean protein, 80.8 for zein and 79.3 for autoclaved casein. Little relationship was found between digestibility values based on feces and those based on digesta from the mid jejunum except that digestibility coefficeints for casein tended to be higher than those of the other protein sources. Protein digestibility coefficients based on digesta from the ileum were similar to those in feces for casein, autoclaved casein and the rapeseed meal samples but considerably lower than those in feces for soybean protein and zein. Apparent digestibility coefficients in the ileum were 70.4, 72.0 and 77.2 for the three rapeseed meal samples compared to 86.3 for casein, 78.2 for autoclaved casein, 70.3 for soybean isolate and 60.8 for zein. Soluble nitrogen in digesta from the mid jejunum and ileum was significantly ($P<0.05$) higher for casein, autoclaved casein and soybean (84 - 90%) than for the rapeseed meals and zein (56 - 72%). Yet neither soluble nitrogen nor free amino N in digesta from the various regions of the gut were related to the eventual digestibility (fecal analyses) of the proteins. There was no indication of lower trypsin and chymotrypsin activities in digesta from the mid jejunum of pigs fed rapeseed meal compared to those fed the other protein sources. Trypsin activity appeared to be proportional to the nitrogen content of the digesta.

Endogenous nitrogen accounted for 75 - 85% of total nitrogen in

digesta from the various regions of the intestinal tract and feces of pigs fed casein compared to 28 - 50% for the other protein sources. However, dilution of digesta and feces by endogenous protein was insufficient to mask major differences in amino acid composition of the dietary proteins.

Marked modifications in amino acid pattern were observed between the ileum and feces; the proportion of proline, glycine and glutamic acid decreased whereas the proportion of alanine, methionine, isoleucine, leucine, tyrosine and phenylalanine increased. These changes were probably the result of bacterial fermentation as indicated by a rapid decrease in the soluble nitrogen between the ileum and caecum and the presence of diaminopimelic acid (DAP) in the feces of pigs. Calculations based on DAP suggested that 40 to 56% of the fecal nitrogen was of bacterial origin.

True amino acid digestibility coefficients for the rapeseed meals were consistently lower than for casein, soybean isolate and zein but did not differ appreciably for several amino acids from those of autoclaved casein. However no general pattern was found for the digestibilities of individual amino acids although coefficients tended to be highest for glutamic acid, arginine and histidine and lowest for methionine and alanine regardless of dietary protein source. There was fairly close agreement between the ileum and feces in digestibility coefficients for lysine, histidine, arginine, alanine, isoleucine, leucine, tyrosine and phenylalanine. However, there was considerable discrepancy between these two sites in the digestibilities for proline, glycine, glutamic acid, aspartic acid, threonine and serine. This discrepancy was particularly marked in the case of soybean isolate and zein. These findings suggest that some amino acids are more

susceptible to breakdown than others and that the extent of breakdown varies among proteins. Thus although fecal analysis appeared to give reasonable estimates of the availability of individual amino acids for a highly digested protein, such as casein, and for autoclaved casein and rapeseed meal, relatively poor estimates were found with proteins such as soybean isolate and zein, which appeared to undergo considerable fermentation in the large intestine.

The third experiment (Experiment III) investigated the effect of partial removal of the hull fraction by air classification on the digestibility of rapeseed meal protein. A significantly ($P<0.05$) higher digestibility was found for low-fiber rapeseed meals whereas fiber had no effect on the digestibility of soybean protein. Coefficients of apparent digestibility averaged 75.7 for the regular rapeseed meals, 79.8 for the low-fiber rapeseed meals and 83.4 and 83.5 for soybean meal and soybean isolate, respectively. No significant differences were found among protein sources (regular and low-fiber) for protein digestibility coefficients in the mid jejunum and ileum. However, a significant ($P<0.05$) improvement in digestibility was found between the ileum and feces for pigs fed soybean protein either as the meal or the isolate and for two of the low-fiber rapeseed meals. These results suggest that the fiber-rich hull fraction interfered with fermentation of the nitrogenous components or increased the rate of transit through the large intestine.

The high content of aspartic acid in soybean protein and proline in rapeseed protein was reflected by significantly ($P<0.05$) higher levels of these amino acids in the digesta and feces of pigs fed these proteins. However, the influence of dietary amino acids on the amino acid pattern of digesta was difficult to assess because no major

differences in the amino acid composition exist between soybean protein and rapeseed meal.

A significant ($P<0.05$) improvement occurred between the ileum and feces in the apparent digestibilities of proline, glycine, glutamic acid, aspartic acid, threonine and serine. The improvement in digestibility was higher with pigs fed soybean isolate, soybean meal and the low-fiber Bronowski meal than for those fed the regular or low-fiber B.campestris meal. Feed intake was significantly ($P<0.05$) higher for pigs fed soybean, B.campestris and Bronowski diets than for those fed the B.napus diets. However, nitrogen retention expressed as percent of the nitrogen intake or percent of nitrogen absorbed was significantly ($P<0.05$) higher with the rapeseed meal diets than with the soybean protein diets.

Experiment IV dealt with _in vitro_ pepsin, pancreatin and pepsin-pancreatin digestions of the protein sources used in Experiment II and a sample of soybean meal. Little agreement was found between _in vitro_ digestion and apparent digestibility of the proteins although the pepsin-pancreatin digestion of casein, autoclaved casein and zein bore several similarities to the apparent digestibility coefficients for these proteins. Digestion of casein by pancreatin appeared particularly rapid compared to the other protein sources. Pre-digestion of soybean meal and rapeseed meal with pepsin had an appreciable effect on digestion of these proteins. Neither solubility of protein nor rate of enzymatic hydrolysis appeared to completely explain the lower _in vivo_ digestibility of rapeseed meal compared to other protein sources, although there is some indication that the poor solubility of zein was responsible for its poor digestibility.

In conclusion, results of the present study indicate that the digestion and absorption of rapeseed meal is lower than that of casein

but equivalent to that of soybean protein and that the lower digestibility of rapeseed meal than soybean meal is due to the greater fermentation of the latter in the large intestine.

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Appendix, Table 1. Duncan's Multiple Range analysis of percent of total protein, soluble protein and amino nitrogen in digesta from pigs fed rapeseed meal and other protein sources.

Source of Nitrogen	Source of Digesta	Protein Source							
					Rapeseed				
		Casein	Autoclaved Casein	Soybean Isolate	Zein	RSM-1	RSM-4	RSM-5	Protein Free
Total Protein[1] (%)	Stomach	19.7 bcd	20.7 abc	21.7 ab	23.1 a	17.7 de	17.5 de	18.1 cd	2.9 e
	Mid jejunum	15.3 d	26.0 ab	24.7 abc	29.5 a	19.7 cd	21.4 bcd	19.9 cd	9.8 e
	Ileum	11.5 cd	21.7 b	22.6 ab	27.1 a	15.1 c	13.6 c	11.7 cd	6.2 d
	Caecum	9.7 d	19.1 a	17.8 ab	14.3 c	15.7 bc	15.7 bc	15.0 c	5.0 e
	Rectum	10.0 d	20.0 a	16.9 b	12.9 c	16.5 b	17.5 ab	15.4 bc	6.4 e
	Feces	8.5 d	18.8 a	14.8 c	17.4 ab	17.5 ab	16.9 abc	15.9 bc	5.7 e
Soluble Protein[2] (%)	Stomach	46.1 b	33.1 bc	44.2 b	26.6 c	45.6 b	39.8 b	42.0 b	75.0 a
	Mid jejunum	89.7 a	90.2 a	88.2 a	72.3 b	65.7 bc	66.4 c	60.2 bc	88.7 a
	Ileum	83.9 abc	87.8 ab	88.5 a	71.0 d	63.5 de	56.2 e	63.4 de	75.3 bcd
	Caecum	45.3 b	74.6 a	66.6 a	30.7 c	38.5 bc	31.7 c	33.7 c	71.5 a
	Feces	29.1 bc	45.5 a	50.6 a	17.2 d	23.1 bcd	18.8 d	20.1 cd	31.1 b
Amino Nitrogen[3] (%)	Stomach	8.5 a	9.7 a	7.6 a	8.8 a	8.2 a	6.5 a	8.1 a	1.5 b
	Mid jejunum	34.1 a	21.0 bcd	19.8 cd	32.3 ab	24.3 abcd	23.9 abcd	27.3 abc	13.3 d
	Ileum	29.0 b	16.4 c	15.2 c	37.2 a	18.8 c	19.5 c	19.1 c	18.0 c
	Caecum	25.8 ab	21.4 b	14.9 b	35.4 a	25.1 b	23.7 b	23.6 b	18.4 b

[1]Expressed as % of dry matter of digesta.

[2]Expressed as % of total protein of digesta.

[3]Expressed as % of soluble protein.

[4]Values in the same row followed by the same letter did not differ significantly ($P<0.05$).

Appendix, Table 2. Duncan's Multiple Range analysis of percent of total protein, soluble protein and amino nitrogen in digesta from pigs fed regular and low fiber rapeseed meals, soybean meal and soybean isolate.

		Protein Source							
				Rapeseed					
		Soybean		B. campestris		B. napus		B. napus cv. Bronowski	
Source of Nitrogen	Source of Digesta	Meal	Isolate	Meal	Low Fiber	Meal	Low Fiber	Meal	Low Fiber
Total Protein (%)	Stomach	21.0	20.3	17.8	19.0	17.4	17.7	20.9	16.9
		a	ab	c	b	c	c	a	b
	Mid jejunum	25.1	28.5	18.2	19.5	19.2	23.0	23.0	22.1
		a	ab	d	cd	cd	bc	bc	bcd
	Ileum	21.1	24.8	13.5	14.6	12.9	19.1	19.6	17.4
		ab	a	d	cd	d	b	b	bc
	Caecum	20.4	18.5	16.7	17.9	16.2	18.7	18.9	17.0
		a	ab	b	ab	b	ab	ab	ab
	Rectum	20.5	20.7	19.0	17.7	17.0	19.9	22.5	18.7
		ab	ab	ab	b	b	ab	a	ab
	Feces	20.0	16.4	17.8	16.9	17.4	18.0	21.7	20.0
		a	b	ab	b	ab	ab	a	b
Soluble Protein (%)	Stomach	26.7	34.3	34.5	37.5	31.8	36.5	32.3	32.4
		a	a	a	a	a	a	a	a
	Mid jejunum	89.3	92.6	72.4	77.9	75.2	67.3	69.9	75.5
		a	a	b	b	b	b	b	b
	Ileum	89.4	88.2	58.6	64.0	72.5	60.2	69.4	76.8
		a	a	d	cd	bc	d	bcd	b
	Caecum	56.2	72.7	35.2	42.1	35.2	45.7	27.4	44.2
		b	a	cd	c	cd	bc	d	bc
	Feces	36.2	58.5	26.1	26.1	25.5	29.2	21.6	24.2
		b	a	bc	bc	bc	bc	c	bc
Amino Nitrogen (%)	Stomach	7.7	4.3	6.2	6.4	7.4	7.8	7.8	7.6
		a	a	a	a	a	a	a	a
	Mid jejunum	29.3	21.6	24.4	26.5	25.8	26.2	23.2	25.8
		a	a	a	a	a	a	a	a
	Ileum	31.1	17.5	23.5	20.9	16.2	28.9	18.6	25.9
		a	c	abc	bc	c	ab	c	abc
	Caecum	30.9	18.8	21.5	29.7	22.9	23.4	24.9	24.4
		a	a	a	a	a	a	a	a

[1]Expressed as % of dry matter of digesta.

[2]Expressed as % of total protein of digesta.

[3]Expressed as % of soluble protein.

[4]Values in the same row followed by the same letter did not differ significantly ($P<0.05$).

Appendix, Table 3. Percent of amino acid recovery (including NH_3) in diets, digesta and feces with pigs fed rapeseed meal and other protein sources (Experiment II).

Source of Nitrogen	Protein Source						
	Casein	Autoclaved Casein	Soybean Isolate	Zein	Rapeseed RSM-1	Rapeseed RSM-5	Protein-Free
Diet[1]	97.6 ± 2.5	96.5 ± 2.8	97.5 ± 3.5	96.6 ± 5.1	87.7 ± 0.7	86.0 ± 0.3	-
	a	a	a	a	b	b	
Mid jejunum	91.9 ± 4.7	92.2 ± 4.6	94.4 ± 3.5	94.1 ± 1.4	82.1 ± 1.4	81.4 ± 8.4	94.0 ± 3.4
	a	a	a	a	b	b	a
Ileum[2]	77.5 ± 1.6	80.7 ± 3.3	80.0 ± 4.4	82.8 ± 3.6	68.1 ± 4.8	67.0 ± 3.1	79.8 ± 3.4
	a	a	a	a	b	b	a
Caecum[2]	75.7 ± 1.7	74.2 ± 1.4	77.5 ± 6.1	77.8 ± 3.2	68.2 ± 5.1	68.2 ± 4.0	76.0 ± 1.8
	a	a	a	a	b	b	a
Feces[3]	75.8 ± 1.6	75.2 ± 2.1	76.1 ± 1.2	78.3 ± 2.3	71.7 ± 1.2	69.2 ± 2.8	74.7 ± 2.2
	ab	ab	ab	a	cd	d	bc

[1]Duplicate analyses.

[2]Values in the mid jejunum, ileum and caecum are means ± $S.D._{\bar{x}}$ for 3 pigs.

[3]Values in the feces are means ± $S.D._{\bar{x}}$ for 4 pigs.

[4]Any mean not followed by the same letter is significantly ($P < 0.05$) different.

Appendix, Table 4. Percent of amino acid recovery (including NH_3) in diets, digesta and feces with pigs fed regular and low-fiber rapeseed meal, soybean meal and soybean isolate. (Experiment III).

Source of Nitrogen	Protein Source					
			Rapeseed			
	Soybean		B.campestris		B.napus cv. Bronowski	
	Meal	Isolate	Meal	Low Fiber	Meal	Low Fiber
Diet	92.5	97.0	80.5	83.6	90.7	94.1
Mid jejunum	87.7[1] ± 5.2	92.8 ± 4.5	75.8 ± 4.1	75.5 ± 3.0	85.5 ± 2.9	82.5 ± 3.3
	ab[2]	a	c	c	b	b
Ileum	77.5 ± 2.4	78.9[3] ± 3.5	68.4[3] ± 3.8	67.8[3] ± 2.3	69.1 ± 3.1	69.1 ± 4.2
	a	a	b	b	b	b
Feces	77.7 ± 3.3	78.5 ± 2.7	65.7 ± 1.8	68.7 ± 4.4	70.3 ± 2.9	71.7 ± 4.6
	a	a	c	bc	b	b

[1]Mean ± $S.D._x$ for 5 pigs except as indicated by superscript 3.

[2]Any mean not followed by same letter is significantly ($P < 0.05$) different.

[3]Mean ± $S.D._x$ for 4 pigs.

Appendix, Table 5. Covariance analysis between feed intake and nitrogen retention (gm N retained/4 days) for pigs fed 12 different samples of rapeseed meal (Experiment I).

Source of Variation	d.f.	Sum of Squares and Products			Deviation from Regression			
		Σx^2	Σxy	Σy^2	d.f.	S.S.	M.S.	F
Total	71	16,926,877	240,575	6379.6	70	2960		
Within	11	6,222,495	108,768	2321.2	11	525	47.73	1.16
Error	60	10,704,382	131,807	4058.4	59	2435	41.27	

F = not significant.

Appendix, Table 6. Covariance analysis between feed intake and nitrogen retention (gm N retained/5 days) for pigs fed regular and low-fiber rapeseed meal, soybean meal and soybean isolate.

Source of Variation	d.f.	Sums of Squares and Products			Deviation from Regression			
		Σx^2	Σxy	Σy^2	d.f.	S.S.	M.S.	F
Total	38	1178.7	1676.0	3014.5				
Treatment	3	180.7	196.6	571.0				
Fiber	1	157.2	51.8	186.0				
Interaction	3	92.8	362.7	411.3				
Within	7	430.7	611.0	1168.4				
Error	31	748.0	1065.0	1846.1	30	330.1	11.0	
Treatment + Error	34	928.7	1261.6	2417.6	33	703.8		
					3	373.7	124.6	F = 11.3**
Fiber + Error	32	105.2	1116.8	2032.2	31	654.4		
					1	324.3	324.3	F = 29.5**
Interaction + Error	34	840.9	1427.7	2257.5	33	-166.5		
					3	496.7	165.6	F = 15,05**
Within + Error	38	1178.7	1676.0	3014.5	37	634.5		
					7	304.4	43.0	F = 3.95**

**Significant at $P < 0.01$.

Appendix, Table 7. Levels of glucosinolate[1] (aglycone) of rapeseed meals used in Experiment I.

Sample No.	Glucosinolate products (mg aglycone/gm oil-free meal)		
	Butenyl isothiocyanate[2]	Pentenyl isothiocyanate[2]	Oxazolidinethione
1	2.1	0.5	7.9
2	2.6	2.2	2.3
3	3.6	2.8	2.6
43	0.1	Traces	0.2
5	2.5	1.9	2.0
6	1.6	1.0	1.7
7	4.1	3.1	2.6
8	3.6	3.0	2.4
9	3.1	0.9	11.1
10	2.9	1.0	8.4
Mean of B.napus (1, 9, 10)	2.7	0.8	8.7
Mean of B.campestris (all others except sample 4)	3.0	2.3	2.3

[1]Averages of data supplied by laboratories listed in 1st progress report (Giovanetti and Bell, 1971) after hydrolysis of glucosinolates with myrosinase. Without the addition of this enzyme, only trace amounts of glucosinolate products were found, the maximum being 0.3 mg oxazolidinethione/g oil-free rapeseed meal 9.

[2]Data supplied by Youngs (Prairie Regional Laboratory, National Research Council, Saskatoon).

[3]Data from Bell et al. (1971).

Appendix, Table 8. Levels of glucosinolate[1] (aglycone) of rapeseed meals used in Experiment III.

Glucosinolate Products (mg aglycone/ gm oil-free meal)	Source of Rapeseed					
	B.campestris		B.napus		B.napus cv. Bronowski	
	Meal	Low Fiber	Meal	Low Fiber	Meal	Low Fiber
Isothiocyanates -						
Butenyl	2.181	2.503	1.447	2.246	0.106	0.108
Pentenyl	1.551	1.954	0.261	0.413	-	-
Methylsulfinylbutyl	-	0.118	-	-	-	-
Phenethyl	0.404	0.227	0.131	0.372	-	-
Oxazolidinethiones	0.647	1.802	5.761	8.124	0.132	0.062

[1]Glucosinolate determinations were made by Dr. F.W. Hougen, Department of Plant Science, University of Manitoba.