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ENDOGENOUS PROTEIN IN ILEAL DIGESTA: ESTIMATION OF THE MUCIN CONTENT AND AN EVALUATION OF THE ^{15}N-ISOTOPE DILUTION TECHNIQUE

BY

KELVIN ALEX LIEN 

A THESIS SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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BE GENTLE, KIND AND TRUE

Papa Dougall

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **ENDOGENOUS PROTEIN IN ILEAL DIGESTA: ESTIMATION OF MUCIN CONTENTS AND AN EVALUATION OF THE ^{15}N-ISOTOPE DILUTION TECHNIQUE** submitted by **KELVIN ALEX LIEN** in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** in **NUTRITION**.

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Dated: Dec 13, 1994

DEDICATION

To my wife Janet for all her love and dedication
and her patience and support

To my daughter Christine, who has brought a new
ray of sunshine into my life

To my brother Dale, who has forever been my source inspiration

ABSTRACT

The objectives of these studies were to: 1) determine the contribution of mucin to endogenous protein in ileal digesta, 2) determine the effect of fiber consumption on the output of mucin at the distal ileum and 3) evaluate the ^{15}N-isotope dilution technique for determining the recovery of endogenous protein in ileal digesta.

The output of mucin in ileal digesta of pigs, average initial BW 55 kg, fed a protein-free diet ranged from 5.3 to 6.0 g/d, originating primarily from the small intestine. With the exception of threonine (28.0 to 34.4%), serine (13.2 to 16.3%) and proline (7.2 to 23.8%), mucin represented less than 10% of endogenous amino acids but was the major source of endogenous carbohydrates.

The effect of fiber consumption on mucin output in ileal digesta was studied in human ileostomates consuming total enteral nutrition formulas with graded contents of soy fiber and in pigs consuming increasing amounts of pea fiber with a wheat-based diet. Mucin output was not influenced by the consumption of 1.1 to 33.7 g soy fiber/d, however, it was higher in males than in females. Mucin output increased from 6.0 to 7.6 g/d as pea fiber consumption was increased from 0 to 240 g/d. Abrasion, as influenced by the amount of undigested dietary residuals, may be a primary factor determining the output of mucin in ileal digesta.

An evaluation of the ^{15}N-isotope dilution technique was carried out in three pigs fitted with re-entrant cannulas at the distal ileum and continuously administered [^{15}N]leucine intravenously for 8 d. Enrichments in N were similar in all pools, however, enrichments in amino acids in the TCA-soluble fraction of plasma were at least twice that in the other pools, suggesting that the ^{15}N-enrichment in the actual precursor pool for endogenous N (plasma free amino acids, urea and ammonia) is diluted by a large fraction of N-containing compounds in the TCA-soluble fraction of plasma that are not directly related to endogenous N entering the intestinal lumen. As a result, the contribution of

endogenous to total N in ileal digesta is overestimated.

Enrichments in individual amino acids demonstrate that; a) enrichments in endogenous amino acids are overestimated in blood samples taken only at the time of feeding; b) the TCA-soluble fraction of plasma is a sufficient precursor pool; and c) bacteria do not modify ^{15}N-enrichments in ileal digesta. Endogenous amino acid recoveries determined by the dilution of ^{15}N in individual amino acids were lower ($P < 0.05$) than those determined with the ^{15}N-isotope dilution technique and higher, although not significantly, than those determined by feeding protein-free diets.

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LIST OF ABBREVIATIONS NOT DEFINED IN THE TEXT

Abbreviation	Definition
BW	Body Weight
°C	Degrees Celsius
CP	Crude Protein (N x 6.25)
d	Day
DM	Dry Matter
g	Gram
g	Gravity
h	Hour
kg	Kilogram
i.d.	Inside Diameter
L	Liter
µg	Microgram
µL	Microliter
mg	Milligram
mL	Milliliter
min	Minute
M	Molarity (mol/L)
N	Nitrogen
n	Number
P	Probability
vol	Volume
wt	Weight

CHAPTER I

GENERAL INTRODUCTION

Assessment of the contribution of endogenous protein to total protein recovered at the distal ileum is an important aspect in determining the true digestibility of dietary ingredients. Information concerning the recovery of endogenous proteins in ileal digesta, particularly those from individual sources, can provide important insight into the effect of dietary ingredients in the digestive tract.

A. Endogenous Protein Secretion

Endogenous proteins originate from digestive enzymes (salivary, pancreatic and mucosal), gastric and bile secretions, from sloughed epithelial cells and secretions from the intestinal mucosa. The secretion of endogenous protein from these sources have been studied and the results summarized in recent reviews (Low, 1982a; Souffrant, 1991). Although several of these studies have demonstrated that dietary components can influence endogenous secretions, few studies have focused on the recovery of these proteins at the distal ileum. Results reported by Low (1982b) suggest that the consumption of diets based on barley-wheat-fishmeal, groundnut or casein may have an influence on the recovery of pancreatic proteases at the distal ileum, independent of their effects on enzyme secretion.

Studies concerning the secretion of endogenous protein from individual sources play an important and integral role in our understanding of the effect of dietary ingredients on protein digestion and absorption and on the digestive tract itself. However, a measure of the relative recoveries of different endogenous

proteins at the distal ileum is also important since dietary ingredients might also interact with endogenous proteins in the intestinal lumen in manner that might influence their recoveries at the distal ileum.

Endogenous nitrogen derived from the gastrointestinal mucosa is particularily important since it is estimated to represent 64 to 83% of the total daily endogenous nitrogen entering the digestive tract (Low, 1982a; Souffrant, 1991), which is consistant with its high metabolic activity. Approximately 43% of whole body protein synthesis occurs in the liver and gastrointestinal tract, despite the fact that these organs represent only 14% of whole body protein (McNurlan and Garlick, 1980). Rates of protein synthesis in the mucosa are twice that in the serosa (Garlick et al., 1980; Attaix and Arnal, 1987) which is consistant with its secretory role. While McNurlan et al. (1979) have suggested that as much as 50% of the protein synthesized in the jejunal mucosa might be attributed to replacement of lost epithelial cells, DaCosta et al. (1971) reported that only 8 to 15% of the protein lost from the small intestine was derived from this source.

Considering the contribution of the gastrointestinal mucosa endogenous protein secretion, the recovery of mucin in ileal digesta is important for two reasons. As the primary component of mucus, the protective lining of the gastrointestinal tract, mucins are exposed to all of the chemical and physical forces of digestion. Proteolytic digestion of the mucus layer, augmented by physical abrasion, is proposed to be the primary reason for the presence of mucin in the intestinal lumen (Allen, 1981). In addition, while endogenous N is estimated to be 70 to 80% reabsorbed (Low, 1982a; Souffrant et al., 1986; Krawielitzki et al., 1990), little digestion of mucin occurs prior to the large intestine (Hoskins, 1984). Therefore, mucin could potentially represent a considerable proportion of the endogenous protein recovered at the distal ileum. The second reason for an interest in the recovery of mucin in ileal digesta is that changes in outputs of mucin at the distal ileum might not only reflect the effect of the diet on the mucus gel but also on the intestine itself.

B. Functions of Mucus

Mucus is a large molecular weight glycoprotein that covers the entire lumenal surface of the gastrointestinal tract. As such, several functions have been attributed to it, primary related to protection of the underlying epithelium (Neutra and Forstner, 1987; Turnberg, 1987; Lamont, 1992). Mucus, in conjunction with bicarbonate, protects the epithelium from vigorous digestive processes and corrosive gastric juices by creating an unstirred layer and by acting as a diffusion barrier, preventing large molecular weight compounds (such as proteolytic enzymes) from reaching the epithelium. Mucus traps toxins and prevents infection by trapping bacteria. Adherrent mucus, along with soluble mucus in the intestinal lumen, act as a lubricant, providing protection from mechanical damage caused by the passage of food. Mucus also plays an important role in the digestive process by creating a digestive zone in which digestive enzymes are immobilized near the epithelial surface, preventing the rapid removal of enzymes by peristalsis and places them in a more favorable position for the digestion and absorption of substrates.

C. Structure of Mucus

An overview of mucin structure and composition in healthy individuals is presented from current reviews (Allen, 1981; Allen, 1984; Allen et al., 1984; Forstner and Forstner, 1986; Neutra and Forstner, 1987; Mantle and Allen, 1989; Lamont, 1992).

The major components of mucus gels are the large molecular weight glycoproteins, 2 x 10^6 daltons, called mucins. Mucins are comprised of four subunits (mucin subunits), weighing approximately 5 x 10^5 daltons, that are linked by disulphide bonding and arranged into the 3-dimensional polymeric structure necessary for gel formation. Mercaptoethanol or proteolytically reduced

glycoprotein subunits lack gel forming properties (Allen et al., 1984; Bell et al., 1985; Pearson et al., 1986). Two different models have been proposed for the polymeric structure of mucin which tends to exist in a flexible spheroidal shape in solution. Allen et al. (1981) proposed a four bladed windmill structure in which the protein backbones of all four mucin subunits are linked in a common area, the 'naked' region. Carlstedt and Sheehan (1984) proposed that mucin resembled a 'coiled thread', a long peptide containing several glycosylated and nonglycosylated regions.

Mucin polymers overlap and are joined noncovalently to provide the structural basis of the gel. These noncovalent interactions are strong enough to resist osmotic pressure and solubilization, but are not strong enough to resist gel spreading or mechanical disruption (Bell et. al. 1985). Mucus gels are filled with large volumes of water, up to 95% by weight, and organic constituents such as lipids, proteins, ions and enzymes that are probably acquired from epithelial secretions and from exfoliated and disrupted cells (Neutra and Forstner 1987).

Mucins consist of a protein backbone surrounded by oligosaccharide chains resembling a 'bottle-brush' structure (Allen, 1981). Regional differences in the challenges faced by gastrointestinal mucins are reflected in their compositions. In addition, there is a large degree of microheterogeneity in mucin composition from the same region of the gastrointestinal tract. However, they do have several features in common. Mucins are characterized by a high carbohydrate content, typically over 80% by dry weight, and a relatively low protein content, 15 to 20% (Table I-1). Sulphate represents 2 to 7% of mucin molecules. The carbohydrate fraction consists of galactose, fucose, N-acetylgalactosamine (GalNAc), N-acetylglucosamine (GlcNAc) and sialic acid only. Uronic acids, typical of connective tissue proteoglycans, mannose, common in serum and epithelial glycoproteins, and glucose are not components of mucin glycoproteins.

Mucin carbohydrates are arranged into linear or branched oligosaccharide chains, varying in length from two to twenty-two sugars and are always linked (O-glycosidically) via GalNAc to the hydroxyl of either serine or threonine in the

protein core. Chain elongation occurs by alternating the addition of GlcNAc and galactose. In gastric mucin chain elongation from GalNAc begins with galactose, whereas in the small intestine it begins with GlcNAc. Branches develop by glycosidic bonding of GlcNAc to either galactose or core GalNAc. Sialic acid and fucose are always found in the terminal position at the nonreducing ends of main or branched chains. The arrangement of terminal oligosaccharides specify the ABH antigens of the ABO blood group system. Chains ending in GalNAc are type A, those ending in galactose are type B and chains terminating in fucose α1-2 galactose are type H. Acidity of mucin glycoproteins are conferred by the sialic acid, approximately 2% and 18% in gastric and intestinal mucin, respectively, and sulphate, 3 to 5% of the glycoprotein, contents. Despite the well defined sequence of oligosaccharide chains the extent of completeness and the number of chains per molecule varies (Allen 1981, Allen 1984), resulting in a large degree of polydisparity.

Mucin contains at least two distinct protein regions. The glycosylated region of the mucin molecule, representing more than 95% of the glycoprotein, is termed "native" mucin. The protein in this region, accounting for about 65% of the total protein in mucin, is rich in serine, threonine and proline, 40 to 70 mol/100mol of native mucin protein (Table I-1). Threonine and serine provide attachment sites for the oligosaccharide chains. The high proline content may play a role in maintaining a particular conformation in the protein core, allowing carbohydrate chains to be packed very closely (Forstner and Forstner 1986). It has been suggested that in pig gastric mucin one in every three or four amino acids carries a carbohydrate chain (Allen 1981). The tight packing of oligosaccharides make this region relatively resistant to proteolytic attack.

The second region, based on its accessibility to proteolytic attack, is the nonglycosylated or "naked" region, representing approximately 35% of the total protein or 4-5% of the total glycoprotein. This region has an amino acid composition similar to that of an average globular protein, but is particularily enriched in cysteine, consistant with its role in the formation of the polymeric

structure of mucus via the joining of mucin subunits by disulphide bridges. A third region, the link region, has also been identified (Neutra and Forstner, 1987; Mantle and Allen, 1989).

The composition of gastric and intestinal mucins from pigs determined in a variety of studies are presented in Table I-1. These data demonstrate a relative consistancy in the carbohydrate composition from different studies, despite the overall microheterogeneity of mucin in different regions of the gastrointestinal tract. The composition of mucin carbohydrates as influenced by the blood type of individuals is demonstrated in Table I-2. The most distinguishing feature of these compositions is the relative ratios of GlcNAc and GalNAc in gastric versus intestinal mucin. The GalNAc content is higher in intestinal mucin as a result of the shorter oligosaccharide chains, up to 8 carbohydrate residues per chain compared to 19 in gastric mucin.

D. Degradation of Mucin in the Stomach and Small Intestine

The mucus gel is not static, it is a dynamic balance between erosion and secretion. While the protective capacity of mucus is a function of the depth and quality of the gel, its effectiveness in vivo is dependent on the ability of the underlying epithelium to replace eroded portions. Proteolysis, augmented by the mechanical forces of digestion, has been proposed to be the primary reason for the presence of mucin in the intestinal lumen (Allen, 1981).

Several studies have demonstrated proteolytic digestion of mucus into component mucin subunits by pepsin, pronase, papain and trypsin (Scawen et al., 1977; Pearson et al. 1980; Mantle et al., 1981; Laszewicz et al., 1985). Similar results have been obtained from studies in vivo. Allen et al. (1980) investigated the effects of peptic digestion of mucus by analyzing the relative amounts of degraded (low molecular weight subunits) and undegraded mucus glycoprotein in human gastric washouts. While 70% of mucin in the washouts was degraded,

mucin obtained by scraping the mucosa of gastrectomy patients was 79% undegraded. Mucus acquired by scraping the stomach and intestine of gastric and duodenal ulcer patients had high amounts of degraded glycoprotein, 65.1 and 50.2%, respectively, compared to the normal level of 33.4% in pancreatoduodenectomy patients, although there were no differences in the mean amount of glycoprotein between the three groups (Younan et al., 1982). Sellers et al. (1989) recently demonstrated a correlation between the extent of polymerization of mucins and the strength and stability of mucus gels. Allen et al. (1990) illustrated this relationship in the context of the mucus gel in ulceration, indicating that proteolytic degradation weakens mucus gels and them more susceptable to the physical forces of digestion. Incubation of mucus glycoprotein in various segments of the small intestine results in a decrease in viscosity in conjunction with degradation to mucin subunits (Ofosu at al.,1978). Conversely, the introduction of 0.75 to 3 mg pepsin into the stomach of rats causes a linear increase in the recovery of mucin (Munster et al., 1987). In the absence of pancreatic proteases there is reduction in the turnover rate of many of the large molecular weight proteins of the intestinal brush border (Alpers, 1984), further demonstrating a role for lumenal proteases in regulating mucosal protein synthesis.

Increased pepsin concentration is positively correlated with glycoprotein output (Allen et al., 1980; Munster et al., 1987) and the amount of degraded subunits in samples (Allen et al., 1980; Laszewicz et al., 1985). Peptic degradation of mucus is reduced in the presence of ethanol, a pepsin denaturant (Laszewicz et al., 1985). It is, therefore, proposed that proteolytic enzymes continuously erode mucus gels throughout the gastrointestinal tract. Degradation of mucus is believed necessary to facilitate the transport of materials through the gel layer (Bandurko et al., 1985).

Proteolysis is associated with a general loss, 20 to 30%, of amino acids, except for threonine, serine and proline, and little (< 1%) or no loss of carbohydrate (Table I-1). Overall recovery of mucin is greater than 95%.

Exhausive proteolysis is without further effect (Mantle et al., 1981; Lee et al., 1987). The oligosaccharide chains, therefore, protect the protein of native mucin making it resistant to further proteolysis until their removal. According to Hoskins (1981) and Variyam and Hoskins (1983) at least 50% of the carbohydrates must be removed before any degradation of native protein occurs.

Neither bile (Allen et.al. 1984, Bell et.al. 1985) nor luminal acid have an effect on mucus degradation (Bell et al., 1985). In addition, mucus gels contain a large content of contaminant materials including lipids and proteins which have been demonstrated to strengthen mucus gels, making them more resistant to proteolysis (Sellers et al., 1983; Lee et al., 1987). It is assumed, therefore, that mucin subunits go largely undigested until they reach the large intestine (Variyam and Hoskins, 1983; Hoskins, 1984; Forstner and Forstner, 1986).

E. Degradation of Mucin in the Large Intestine

The degradation of mucin occurs largely by bacteria in the large intestine and the circumstances of this degradation further imply that little digestion of mucin occurs in the small intestine. Clamp and Gough (1991) reported that while glycoprotein represented about 15% of dry matter in ileostomy effluent, only traces were found in feces. Analysis of luminal contents of autopsied humans revealed regional differences in mucin constituents that were consistant with microbial degradation (Vercellotti et.al. 1977). These observations are consistant with a high content of mucin glycoproteins in cecal digesta from germ-free, but not conventional, rats (Lindstedt et al., 1965; Hoskins and Zamcheck, 1968). Prizont and Konigsberg (1981) demonstrated the digestion of glycoproteins from the feces of bacteria-free rats in supernatants from the cecum of coventional rats.

Variyam and Hoskins (1981) incubated hog gastric mucin in human fecal extracts and in anaerobic fecal culture and found that after 48 hours only 65 to 90% of mucin hexoses and 30 to 50% of mucin protein was degraded in fecal

extracts while in anaerobic cultures 20 to 81% of the protein and 89 to 99% of the sugars were degraded. After 96 hours of incubation in two fecal cultures between 40 and 50% of the mucin protein and 93 to 97% of hexoses were degraded (Variyam and Hoskins, 1983). In a study by Miller and Hoskins (1981) mucin hexose degradation exceeded 90% in more than half (61%) of the fecal cultures while mean protein degradation was only 65% after 48 hours incubation.

A variety of glycosidases, produced exclusively by enteric bacteria are required for the complete degradation of mucin oligosaccharide chains. These enzymes are primarily extracellular exoglycosidases that cleave terminal monosaccharides one at a time from the nonreducing end of oligosaccharide chains. A high degree of substrate specificity is determined by the monosaccharide to be cleaved, its anomeric configuration and the location of its glycosidic linkage to the next sugar (Hoskins, 1984). Evidence from Hoskins et al. (1983) and Bayliss and Houston (1984) indicate that a combination of bacterial species, each having a different set of enzymes, may be required for the complete degradation of mucin glycoproteins. However, of the 300 to 500 taxonomically different kinds of bacteria found in feces only a small portion, approximately 1%, are capable of degrading mucin glycoproteins (Miller and Hoskins, 1981; Bayliss and Houston, 1984; Stanley et al., 1986).

The basis for resistance of native mucin protein to proteolytic digestion in the small intestine is a lack of susceptable bonds resulting from hindrance by the oligosaccharide chains (Hoskins, 1984). Removal of the oligosaccharides requires a complicated sequence of enzymes and occurs over a considerable length of time, further indicating that little, if any digestion of mucin occurs prior to the large intestine.

F. Effect of Diet on Mucin Secretion

Few studies have examined the the effect of diets or dietary factors on

mucin secretion. However, since mucin secretion is stimulated by many of the same neural and hormonal factors that control digestive processes (Neura and Forstner, 1987; Mantle and Allen, 1989), the diet might be expected to have considerable consequences on mucin secretion. In addition, the secretion of proteolytic enzymes is also stimulated by these same secretagogues (Hersey, 1987; Solomon, 1987). Since mucin output (eg., Kowalewski et al., 1976; Allen et al., 1980) and the proportion of mucin subunits (Allen et al., 1980) is increased in conjunction with pepsin following the administration of stimulants, Mantle and Allen (1989) have suggested that the effects of some of these stimulants may be modulated by their effect on proteolytic enzyme secretion.

The consumption of food increases mucus synthesis. The synthesis of gastric glycoproteins is reduced in fasted rats (Dekanski et al., 1975; Ohara et al., 1984). Kowalewski et al. (1976) reported a 50% increase in glycoprotein carbohydrate output in canine heidenhain pouches following the consumption of a meal, however, it is possible that this results from proteolytic degradation, since pepsin also increased by 300%. Reducing food intake in rats to one-half their normal daily consumption results in a dramatic decrease in intestinal mucus production (Sherman et al., 1985).

Although the activities of proteolytic enzymes in pancreatic juice do not appear to be influenced by diet when considered on a daily basis (eg., Low, 1982b; Zebrowska et al., 1983), studies by Scheeman suggest that some diets, particularly those enriched with fiber, can have an effect on the activities and distribution of proteolytic enzymes in the intestinal lumen which may influence the degradation of mucus gels. Trypsin and chymotrypsin activities were higher in intestinal contents and lower in mucosal homogenates of rats fed wheat bran compared to those fed a fiber-free diet (Schneeman et al., 1982) but the opposite was true for pectin (Forman and Schneeman, 1980). Guar gum increases total protease activity in the intestine (Poksay and Schneeman, 1983). Farness and Schneeman, (1982) reported that oat bran, pectin and cellulose increased small intestinal peptidase activities in rats by 12%, 159% and 48%, respectively. Pepsin

was doubled in duodenal digesta from pigs fed a high fiber diet, barley-soya bean, versus a low fiber diet, starch-casein-cellulose (Zebrowska et al., 1983).

Adaptation to diets high in fiber induce structural and morophological (Vahouny and Cassidy, 1986) and cytokinetic (Jacobs, 1986) changes in the digestive tract that are consistant with an overall capacity for higher mucin secretion, i.e. more mucin secreting cells as a result of increased surface area and a larger intestinal tract. However, there is little indication as to the immediate effect of fiber on mucus secretion. Vahouny et al. (1985) observed increased mucin secretion in rats fed diets containing cellulose and wheat bran supplemented diets compared to those fed a fiber-free diet. The consumption of citrus fiber, but not guar gum increases mucin secretion in the stomach of rats (Satchithanandan et al., 1990). In addition, citrus fiber and wheat bran increase mucin secretion in rat small intestines but guar gum and carrageenan do not (Satchithanandan et al. 1989, 1990). These studies suggest that the abrasive action of digestive contents, i.e. insoluble versus soluble fibers, may have an important influence on the amount of mucin entering the large intestine.

It is apparent that the consumption of different diets will have an influence on the secretion of mucin in both the stomach and small intestine, indirectly via their influence on the digestive processes and directly through their interaction with the mucus gel. Since the depth of the mucus layer is the result of a dynamic balance between erosion and synthesis, measuring the mucin in this layer may not provide a full account of the effect of dietary treatment on the gastrointestinal mucosa. This is particularily true since animals typically undergo a 24 h fast prior to such measurements (Vahouny et al., 1985; Satchithanandan et al., 1989, 1990). Thus a measure of mucin output may be more suggestive of the effect of dietary treatments on the intestinal mucosa, although this in itself is not completely indicative (Mantle and Allen, 1989). Estimating the recovery of mucin in ileal digesta would also be beneficial since the presence of this glycoprotein in ileal digesta has often been implicated in the low ileal digestibilities of some amino acids, particularily threonine, observed with many feedstuffs (eg., Sauer

and Ozimek, 1986).

G. The ^{15}N-Isotope Dilution Technique

Estimates of the recovery of endogenous protein from different sources at the distal ileum is necessary for a complete understanding of the effect of dietary treatments on digestive processes. However, an assesment of the contribution of total endogenous protein to protein recovered at the distal ileum is also required to determine the true value of dietary ingredients. Two methods are traditionally used to estimate endogenous protein, feeding protein-free diets and the regression method. These methods assume that endogenous protein is constant, on a dry matter intake basis and, therefore, do not provide a means of determining the effect of different diets or dietary nutrients on the recovery of endogenous protein at the distal ileum.

As yet, the best method for determing the contribution of endogenous to total protein in ileal digesta appears to involve the use of ^{15}N-isotope dilution techniques (Souffrant et al., 1986; de Lange et al., 1990) since direct quantitation of endogenous protein is possible. With the ^{15}N-isotope dilution technique animals are labelled via a continuous intravenous infusion of a labelled amino acid, ^{15}N-leucine. Since plasma free amino acids are assumed to represent the precursor pool for endogenous protein synthesis, the plateau ^{15}N-enrichment excess in the TCA-soluble fraction of plasma is assumed to be similar to that of endogenous protein in ileal digesta (Souffrant et al., 1981; 1986). Dilution of the ^{15}N-enrichment in digesta, relative to that in the TCA-soluble fraction of plasma is a measure of the contribution of endogenenous to total protein in ileal digesta. Digestibilities determined with ^{15}N-isotope dilution techniques are referred to as real digestibilities (Low, 1982a).

The ^{15}N-isotope dilution technique has been used in several recent studies to estimate real protein digestibilities of feedstuffs for swine (Souffrant et al., 1981;

de Lange et al., 1990, 1992; Huisman et al., 1992; Mosenthin et al., 1993). The results from these studies suggest that endogenous protein recovery at the distal ileum is relatively constant when related to dry matter intake (Souffrant et al., 1991), although approximately 50% higher than that determined by feeding protein-free diets (eg., de Lange et al., 1989). However, estimates of real digestibilities approaching 100%, 99.0, 94.2 and 97.5% for wheat, barley and soybean meal, respectively (de Lange et al., 1990), suggest that the contribution of endogenous to total protein in ileal digesta is overestimated with technique. This is supported by the observation that real digestibilities of several amino acids exceed 100% when calculated from the recovery of endogenous protein, assuming a constant endogenous protein composition (de Lange et al., 1990).

Recognizing that ^{15}N-enrichments would not be the same in all N-containing compounds, de Lange et al. (1992) attempted to estimate endogenous protein recovery more directly, i.e. by dilution in amino acids, as opposed to N. However, when the ^{15}N-leucine or the ^{15}N-isotope dilution techniques were used variable results were obtained (de Lange et al., 1992). Recoveries of endogenous protein determined with the ^{15}N-leucine isotope dilution technique, 7.1 to 11.0g/kg DM intake, were considerably lower than those obtained with the ^{15}N-, 25.5 to 30.5g/kg DM intake, and ^{15}N-isoleucine, 21.8 to 24.9g/kg DM intake, isotope dilution techniques. Recoveries of endogenous protein determined with the ^{15}N-leucine isotope dilution technique were even lower than those obtained by feeding protein-free diets (de Lange et al., 1989). These authors raised two very important points in trying to address this apparent inconsistancy, the effect of pattern of blood sampling and the validity of the TCA-soluble fraction of plasma as a precursor pool for endogenous protein synthesis.

Blood samples are typically taken at the time of feeding, every 8 to 12 h, although digesta are collected continuosly over the entire period between feedings. Amino acids in the TCA-soluble fraction of plasma would be diluted to a lesser extent at feeding than 3 to 5 h after feeding when large quantities of dietary amino acids enter the portal blood (Rerat, 1985). Since endogenous

protein secretion is more-or-less continuous, dilution of ^{15}N would be reflected in ileal digesta, but not the TCA-soluble fraction of plasma. Moreover, catabolism of leucine is reduced in the postabsorptive state (e.g. Elia and Livesey, 1983; Castellino et al., 1987), which is expected to be achieved by 12 h after feeding, thereby reducing the transfer of ^{15}N to other compounds and increasing the difference in ^{15}N-enrichment between leucine and isoleucine. An overestimation of the ^{15}N-enrichment in plasma will result in an underestimation in the recovery of endogenous protein at the distal ileum. This effect will be more prounounced for leucine than for isoleucine. It is apparent, therefore, that more frequent blood samples need to be obtained to better estimate the ^{15}N-enrichment in plasma over the entire period for which digesta is collected.

Results from the study by de Lange et al., (1992) also raised questions as to the validity of using the TCA-soluble fraction of plasma to estimate the ^{15}N-enrichment in endogenous protein secreted into the digestive tract. This is especially important for the proteins derived from the gastrointestinal mucosa since this the largest contributor of endogenous protein secreted into the digestive tract. Several studies suggest that the intestine itself may a more valid precursor pool for proteins secreted into the intestinal tract. Protein synthesis occurs along the entire villus and crypt, while amino acid absorption is restricted to the villus. Studies in which labelled amino acids are infused luminally and intravenously (Alpers, 1972) indicate that luminal amino acids are incorporated into protein largely in the enterocytes on the villus, whereas systemic amino acids are preferentially used by crypt cells. Hirschfield and Kern (1969) demonstrated that luminal amino acids are more readily utilized for protein synthesis in the intestinal mucosa than are systemic amino acids. Incubated rings of rat jejunum accumulate amino acids in substantial quantities and incorporate them into mucosal protein (Bronk and Parsons, 1966). At isotopic steady state, following a primed constant infusion of [^{3}H]leucine for three hour, the specific radioactivity of prosucrase-isomaltase (pro-SI), the direct precursor of sucrase, was intermediate to that of the free amino acid pools of the mucosa and

plasma (Dudley et al., 1992). Furthermore, the relative contributions of systemic and lumenal amino acids to pro-SI synthesis appears is dependent on the physiological state of the animal.

The relative contribution of amino acids derived from the systemic circulation, either directly or indirectly, and from the diet to endogenous protein secreted into the intestinal lumen remains to be elucidated. However, it is clear that intensive utilization of lumenal amino acids for protein synthesis in gut tissue will result in a dilution of labelled amino acids originating from the blood plasma pool, by unlabelled amino acids from the diet. This will result in a lower labelling of endogenous amino acids from the intestinal wall as compared to that in the blood plasma pool (Souffrant et al., 1986). Because of this, the actual contribution of endogenous protein to total protein in the digestive tract, based on the ^{15}N-enrichment excess in the TCA soluble fraction of blood and in digesta will be underestimated. It could also be argued, however, that the large degree of recycling that occurs during the digestive process may make this point moot. That is, the continuous recycling of endogenous and dietary amino acids during the digestive process may make the enrichments in recently absorbed amino acids indistinguishable from those in the TCA-soluble fraction of plasma. Pancreatic proteins rapidly incorporate oral (Leterme et al., 1994) and intravenously (Simon et al., 1983) administered isotopes. A validation of the TCA-soluble fraction of plasma for estimating the ^{15}N-enrichment in endogenous protein in ileal digesta is required.

H. Objectives of This Research

Estimates of the recovery of mucin in ileal digesta will greatly enhance our understanding of the effect the consumption of different diets and dietary constituents on the digestive process. In addition, since mucin is poorly digested in the small intestine, it could represent a considerable proportion of endogenous

protein recovered at the distal ileum. Despite the importance of estimates of the recovery of endogenous proteins from different sources at the distal ileum, reliable estimates of the total recovery of endogenous protein is also required so that assessments of the true value of dietary ingredients can be made. Studies described in this thesis were carried out to achieve the following goals:

1. to estimate the recovery of mucin in ileal digesta of pigs feed a protein-free diet to determine the contribution of mucin protein to total endogenous protein in ileal digesta.

2. to determine the effect of fiber consumption on the daily output of mucin at the distal ileum.

3. to present an evaluation of the ^{15}N-isotope dilution technique for determining the recovery of endogenous protein in ileal digesta by:

   a. determining the effect of dilution in the actual precursor for endogenous N secretion on estimates of the contribution endogenous N to total N in ileal digesta,

   b. determining the effect of the the pattern of blood sampling, different precursor pools and different ^{15}N-isotope dilution techniques on estimates of the recovery of endogenous protein at the distal ileum.

Table I-1. The composition of native and pronase digested gastric and small intestinal mucins from pigs

	Gastric			Intestinal		
	Native (1)	Pronase (1)	(2)	Native (3)	Pronase (4)	(5)
Total Composition (% DM)						
Carbohydrate	78.0	82.1		54.1	63.6	
Protein	15.4	11.4		21.2	15.1	
Sialic acid	2.9	2.9		21.6	17.5	
Sulphate	3.7	3.7		3.1	3.8	
Protein composition (mol/100mol)						
Threonine	18.3	25.3		27.2	34.8	
Serine	18.1	26.1		12.1	15.5	
Proline	16.0	18.5		16.4	21.0	
Rest	47.6	30.1		44.3	28.7	
Carbohydrate composition (mol/100mol)						
Fucose	17.4	16.9	15.5	9.6	14.0	10.5
Galactose	39.9	40.2	36.1	26.5	29.5	27.6
GlcNAc	29.9	29.1	35.3	22.6	19.6	28.9
GalNAc	12.8	13.8	13.1	41.3	36.9	32.9
GlcNAc/GalNAc	2.3	2.1	2.7	0.6	0.5	0.9

(1) Scawen and Allen, 1977; (2) Stanley et al., 1983; (3) Mantle and Allen, 1981 (4) Mantle et al., 1981; (5) Choi et al., 1991.

Table I-2. Selective carbohydrate composition (mol/100mol) of human gastric and intestinal mucins presented by blood group

	Gastric[a]			Intestinal[b]	
	A	O	B	A	H
Fucose	25.6	24.5	22.1	25.5	29.6
Galactose	34.5	37.2	40.3	24.3	31.8
GlcNAc	25.9	27.9	26.6	24.0	26.5
GalNAc	14.1	10.4	11.0	26.2	12.1
GlcNAc/GalNAc	1.8	2.7	2.4	0.9	2.2

[a]Schrager and Oats, 1974; presented by blood-group specificity.
[b]Mantle et al., 1984; presented by blood group.

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CHAPTER II

RECOVERY OF MUCIN FROM ILEAL DIGESTA OF PIGS FED A PROTEIN-FREE DIET

A. Introduction

One of the major impedements to the assessment of the quality of protein-containing feedstuffs is an accurate evaluation of the contribution of endogenous to total protein in ileal digesta for the measurement of true ileal protein and amino acid digestibilities. There is also a scarcity of information on the effects of dietary treatment on the recovery and composition of endogenous protein at the distal ileum. With traditional methods (feeding protein-free diets or the regresssion method), the recovery of endogenous protein is assumed to be constant and related to DM intake. On this basis, these methods do not reflect the influence of diets on the secretion of endogenous protein and thus its recovery at the distal ileum (Souffrant, 1991). Since the gastrointestinal mucosa is the largest source of endogenous protein (Souffrant, 1991), these secretions are of particular importance.

Mucus provides a protective lining for the entire digestive tract and is, therefore, exposed to all chemical and physical forces of digestion (Allen 1981). Proteolysis enhances the solubilization of mucus gels (Bell et al., 1985; Laszewicz et al., 1985). In light of this it has been proposed that proteolysis and physical abrasion are the primary factors responsible for the presence of mucin in the intestinal lumen (Allen, 1981). Mucus secretion is elevated with the consumption of food (Kowalewski et al., 1976; Sherman et al., 1985) and fiber (Vahouney et al., 1985; Satchithanandam et al., 1990). However, evidence suggests that, once in the intestinal lumen, little degradation of gastrointestinal mucus occurs prior to the large intestine, where it is degraded by enteric bacteria (Hoskins, 1984).

Exhaustive proteolysis of mucus *in vitro* (e.g., Scawen and Allen, 1977) and *in vivo* (Ofosu et al., 1978) result in a reduction of only a small proportion of the mucus protein. Mucin could, therefore, represent a considerable portion of the endogenous protein recovered at the distal ileum.

Mucin protein is composed primarily of threonine, serine and proline (Scawen and Allen, 1977; Mantle and Allen, 1981). The presence of mucin in ileal digesta has often been implicated in the predominance of threonine in endogenous protein (e.g., de Lange et al., 1989) and thus its low digestibility in diets for pigs (Sauer and Ozimek, 1986). In addition, degradation of mucin by bacteria in the large intestine would explain the considerable large intestinal disappearance of these amino acids, particularily threonine (Sauer and Ozimek, 1986). It is of interest, therefore, to determine the amount of mucin in ileal digesta of pigs. In addition, feeding protein-free diets to estimate endogenous protein at the distal ileum has been the subject of criticism (e.g., Souffrant, 1991), therefore, the following study also sought to determine the effect of protein status of the pig on the recovery of mucin in ileal digesta.

B. Experimental Procedures

Animals and Procedures

This study was performed using samples collected previously (de Lange et al., 1989), therefore, a detailed description of the experimental procedure has been presented. Sufficient samples for analyses were available for three barrows only, therefore, only three observations for each treatment were obtained for this study. Barrows, with an average initial body weight of 55 kg, were fitted with a simple T-cannula at the distal ileum and a catheter in the external jugular vein. The animals were fed twice daily, at 0800 and 2000, 700 g of a protein-free diet while either a complete mixture of amino acids or saline was administered intravenously for 8 d. The experimental diet consisted of 79.7% cornstarch, 10%

sucrose, 3% canola oil, 3% Alphafloc and a vitamin-mineral premix. Digesta were collected continuously for 24 h on d 8. Following the previous analyses (de Lange et al., 1989), digesta were stored at -30°C.

Chemical Analyses

Crude mucin (CM) was isolated according to modifications of procedures described by Allen (1981) and Miller and Hoskins (1981). Approximately 3 g of freeze-dried digesta was weighed into a 50 mL polystyrene test tube and 25 mL of 0.15M NaCl containing 0.02M sodium azide, maintained at 4°C, was added and samples were homogenized for 1 min using a Polytron homogenizer (Kinematica, Kriens, Switzerland). Homogenized samples were then immediately centrifuged for 30 min at 12,000 x g at 4°C and the aqueous layer decanted into a second 50 mL polystyrene test tube. The aqueous layer was again centrifuged at 12,000 x g for 30 min to ensure the complete removal of insoluble material. Fifteen mL of the aqueous fraction were pipetted into a pre-weighed 50 mL test tube, cooled in an ice-bath, and ice cold ethanol added to a final concentration of 60% (v/v). The samples were allowed to precipitate overnight at -20°C.

The following day samples were centrifuged at 1,400 x g for 10 min at 4°C and the precipitate recovered by decanting the supernatant. The pellet was resolubilized in 15 mL of 0.15M NaCl and cooled in an ice-bath. Precooled ethanol was added to a final concentration of 60% (v/v) and the samples were left overnight to precipitate as before. The CM precipitate was recovered as described previously. Successive rinsing resulted in reduced recovery of CM, without any change in the composition, therefore, samples were rinsed only until a clear supernatant was obtained. The precipitate was resolubilized in 10 mL of water and freeze-dried. Recovery of CM from ileal digesta was linear between 0.5 and 8 g of starting material.

Analyses for nitrogen (N), by the Kjeldahl method (AOAC, 1988), and amino acids, following procedures adapted from Jones and Gilligan (1983), were repeated in ileal digesta samples for this study. Approximately 100 mg of freeze-

dried digesta or 30 mg of CM, was weighed into 10 mL screw cap test tubes. Six mL of 6M HCl was added, the tubes purged with nitrogen and the samples hydrolyzed at 110°C for 24 h. Following hydolysis, 1 mL of water and 200 µL of internal standard were added. Internal standards contained both ß-amino-n-butyric acid and ethanolamine (Sigma Chemical Co., St. Louis, MO) at 25 µmol/mL in water. Amino acid standards were prepared by combining 1 mL of Sigma amino acid standard (2.5 µmol/mL), 200 µL internal standard and 6 mL 6M HCl in a screw cap test tube. Amino acids were analyzed as O-phthaldialdehyde derivitives using a Varian 5000 (Varian Instuments Inc., Walnut Creek, CA) high performance liquid chromatography system. Proline was estimated as its trimethylsilyl derivative by gas chromatography-mass spectrometry using leucine in the samples as a standard.

Carbohydrates were analyzed as their alditol acetates according to procedures adapted from Blakeney et al. (1983) and Kraus et al. (1990). Approximately 50 mg of CM or ileal digesta were treated with 12M sulfuric acid (1.5 mL) for 1 h at room temperature. The solution was diluted to 3M with 4.5 mL of water and the samples hydrolyzed for 1 h at 110 °C. Following hydrolysis, 200 µL of internal standard were added (N-methylglucamine and *myo*-inositol, for amino sugars and neutral sugars, respectively, at 10 mg/mL of distilled water). Aliquots (1 mL) of the acid hydrolysates were cooled in an ice-bath and made basic with the addition of 0.7 mL concentrated ammonium hydroxide. To 100 µL of this, 1 mL sodium borohydride (30 mg/mL in anhydrous dimethylsulphoxide) was added and reduction allowed to proceed for 90 min at 40°C. Excess sodium borohydrate was decomposed with the addition of 200 µL concentrated glacial acetic acid. Following this, 0.2 mL 1-methylimidazole and then 2 mL acetic anhydride were added. The solution was mixed and acetylation occurred at room temperature for 10 to 15 min. Thereafter, 5 mL of water was added to decompose excess acetic anhydride and the mixture cooled to room temperature. Alditol acetates were extracted into 4 mL of dichloromethane by vigorous shaking and the upper aqueous layer removed. The dichloromethane layer was rinsed twice

with 4 mL of water and evaporated to dryness under a stream of nitrogen. Standard sugars and derivitization reagents were obtained from Sigma (Sigma Chemical, St. Louis, MO).

Prior to analysis by gas-liquid chromatography (Varian 3400), the alditol acetates were redissolved in 1 mL of dichloromethane and approximately 0.5 µL of derivitized sample was injected onto a DB-17 fused silica capillary column (0.25 mm i.d. x 30 m; J&W Scientific, Folsom, CA). Helium was used as the carrier gas at a rate of 1.5 mL/min. Injector temperature was programmed from 60°C to 270°C at 150°C/min and maintained for 20 min. Oven temperature was raised at 30°C/min from 50°C to 190°C, held for 3 min, then increased 5°C/min to 270°C and held for 5 min. Detector (flame ionization) temperature was set at 270°C. Peak area integration was made using a Shimadzu Ezchrom Data System (Shimadzu Scientific Instruments Inc., Columbia, MD).

Calculations

Regression equations were derived from the N-acetylglucosamine (GlcNAc) to N-acetylgalactosamine (GalNAc) ratios in purified gastric (Scawen and Allen, 1977) and intestinal (Mantle and Allen, 1981; Mantle et al., 1981) mucins to calculate the contributions of gastric mucin and the GalNAc content. Two formulas were derived, assuming complete native (no proteolytic digestion) or proteolytic (pronase) digested mucin, to estimate the range of mucin output. The relationship between the GlcNAc\GalNAc ratio and contribution of gastric mucin is described by the following regression equations:

Native: $\%\text{gastric mucin} = -80.23 + 183.26x - 71.19x^2 + 11.05x^3$ (1a)

Pronase: $\%\text{gastric mucin} = -82.07 + 188.36x - 74.50x^2 + 11.69x^3$ (1b)

where x = the GlcNAc/GalNAc ratio. The GalNAc content of mucin mixtures is described by the following regression equations:

Native: $\%\text{GalNAc} = 32.30 - 22.74x + 8.83x^2 - 1.37x^3$ (2a)

Pronase: $\%\text{GalNAc} = 34.87 - 25.36x + 10.03x^2 - 1.57x^3$ (2b)

where x = the GlcNAc/GalNAc ratio. Daily output of mucin was calculated from

the estimated GalNAc content and daily outputs of GalNAc in CM or ileal digesta by the following eguation:

$$\text{mucin output} = \text{GalNAc} / \%\text{GalNAc} \quad (3)$$

where GalNAc = GalNAc output in g/day.

Statistical Analyses

To determine treatment effects, data were subjected to statistical analysis using the SAS (1988) General Linear Models procedure. The data were analyzed using a cross-over design, with group (animals that moved together from one period to the next), animals within each group, period and treatment as sources of variation (Peterson, 1985).

C. Results and Discussion

The recovery and composition of CM in ileal digesta of pigs fed a protein-free diet are presented in Table II-1. Daily outputs of CM in ileal digesta were not different ($P = 0.47$) between amino acid (AAI) and saline infused (SAI) pigs. There was, however, a trend ($P < 0.14$) towards an increase in the carbohydrate content resulting in an increase ($P = 0.03$) in the output of total amino acids plus carbohydrates, 4.2± versus 3.5± g/day in AAI pigs. Carbohydrate plus protein accounted for approximately 70% of the CM in both treatments. The remaining portion may be comprised of a variety of components. Sialic acid represents about 18 and 2% of intestinal and gastric mucus, respectively (Allen, 1981). While not analyzed in these samples, moisture and ash in similarily prepared samples from this laboratory ranged from 11 to 16% and 11 to 21%, respectively. The high ash content is likely due to the presence of salt, since no effort was made to remove it from the final product.

The CM preparations contained predominantly those sugars characteristic of mucus glycoproteins; namely fucose, galactose, GalNAc and GlcNAc (Table II-

1). Small quantities of mannose and glucose, common contaminants of mucus preparations (Mantle and Allen, 1981; Snary and Allen, 1971), were also present. The sialic acid contents were not determined. The high protein to carbohydrate ratios, 1.0 and 0.9 in SAI and AAI pigs, respectively, compared to 0.2 to 0.4 in purified mucus (Scawen and Allen, 1977; Mantle and Allen, 1981), is indicative of the presence of contaminant protein.

Threonine, serine and proline comprised approximately 40 mol/100 mol of the amino acids in both CM preparations (Table II-2). The contribution of these amino acids were lower than expected for purified mucus (Allen, 1981), however, similar contributions have been reported in other studies (Snary and Allen, 1971; Forstner et al., 1984; Ohara et al., 1984). Although the low content of threonine, serine and proline confirms the presence of considerable amounts of nonmucin protein, their high contributions, relative to the other amino acids, indicates the predominance of mucin in the water soluble-ethanol precipitable fraction of ileal digesta from pigs fed a protein-free diet.

There were no differences ($P > 0.10$) in the composition of carbohydrates in CM between AAI and SAI pigs. The contents of Galactose and GalNAc were highest (approximately 20% each) followed by GlcNAc (24%) and fucose (14%). The carbohydrate composition was intermediate to that reported for purified pig gastric (Scawen and Allen, 1977) and small intestinal mucins (Mantle and Allen, 1981). Overall, however, the composition more closely resembled that of purified intestinal mucin, particularily with respect to the GlcNAc/GalNAc ratios, 0.82 and 0.78 in SAI and AAI pigs, respectively, suggesting a greater contribution from this source. The GlcNAc to GalNAc ratio is 2.35 for pig gastric mucin (Scawen and Allen, 1977) and 0.55 for pig small intestinal mucin (Mantle and Allen, 1981). The higher contribution of threonine, relative to serine (Table II-2), also indicates a higher proportion of intestinal mucin (Scawen and Allen, 1977; Mantle and Allen, 1981).

Estimates for the daily outputs of soluble mucin are presented in Table II-3, along with the contribution of amino acids and carbohydrates from mucin to

amino acids and carbohydrates in CM, respectively. There was a higher output of soluble mucin in digesta from AAI pigs, 3.4 to 3.6 g/day versus 2.8 to 3 g/day, with more than 70% of the mucin from both treatments originating from the small intestine. Considering the polydisparity in mucus composition (Allen, 1981), the carbohydrate composition of CM was remarkably similar to that of a mixture of gastric and intestinal mucin, with mucin potentially accounting for all of the carbohydrates in CM, except glucose and mannose. However, mucin accounted for less than 40% of the protein; 23 to 37% depending on the degree of proteolytic degradation. The highest contributions of amino acids were for threonine (55%), proline (43%) and serine (34%). The contribution of these amino acids does not change as result of proteolytic degradation because they are predominantly located in the glycosylated region, representing more than 70 mol/100mol of amino acids in this region, and are thus protected from proteolysis (Allen, 1981).

The results presented in Table II-3 demonstrate that although mucin is a predominant constituent of CM, there was a considerable amount of contaminant protein. This protein may represent noncovalently bound protein which is typically associated with mucus gels (Allen, 1981). The presence of this protein has been shown to strengthen mucus gels, making them more resistant to proteolytic degradation (Sellers et al., 1983). Alternatively, it is more than probable that some coprecipitation of soluble proteins had occured.

The fucose content of CM is of particular interest. High mucus synthesis and secretion rates are associated with a lower carbohydrate content, either by a reduction in carbohydrate chain length or the number of chains per molecule (Allen, 1981). The relative contents of the terminal sugars fucose and sialic acid appear to be most affected (Ohara et al., 1984; Forstner et al., 1984). The high fucose content in CM, therefore, suggests that mucus synthesis rates with either treatment were not elevated enough to cause a modification of the mucus carbohydrate composition. Moreover, the carbohydrate content, particularily that of fucose, indicates that little digestion of mucin had occurred, since degradation

of the carbohydrate takes place by stepwise cleavage of individual sugars starting at the terminal end (Hoskins, 1984).

Assuming that all GalNAc in ileal digesta was associated with mucin and that the contributions of gastric and intestinal mucin were similar to that in CM, estimates of the output of mucin in ileal digesta were calculated. These, along with the contributions of mucin carbohydrates and amino acids to their digesta counterparts are presented in Table II-4. Mucin output was only 6% higher in AAI pigs, 5.7 to 6.0 g/day versus 5.3 to 5.6 g/day in SAI pigs. This difference is much less than that observed in CM (about 24%, Table II-3), indicating an increase in the solubility of mucin as a result of the amino acid infusion. Leterme et al. 1994) reported an increase in the amount of N associated with soluble compounds with a molecular weight (MW) exceeding 10^4 daltons in ileal digesta following amino acid infusion in pigs fed a protein-free diet. The MW of mucin subunits is approximately 5×10^5 daltons (Allen, 1981). Mucus gels are readily degraded to mucin subunits by proteolytic enzymes *in vitro* (e.g., Scawen and Allen, 1977) and *in vivo* (e.g., Ofosu et al., 1978), increasing their solubility (Allen, 1981).

The aforementioned discussion suggests an increase in proteolytic activity in the digestive tract in conjunction with the amino acid infusion. Solubilization of mucus is correlated with an increase in proteolytic activity (Bell et al., 1985; Laszewicz et al., 1985). A decrease in N associated with soluble compounds with MW between 10^3 and 10^4 daltons, in conjunction with an increase in N associated with those having a MW lower than 10^3 daltons, following amino acid infusion in pigs fed a protein-free diet (Leterme et al., 1994), also suggests a higher proteolytic activity. The nature of this activity is uncertain since it is clear that, with the exception of proline, there is little effect on the ileal output of endogenous amino acids (de Lange et al., 1989; Leterme et al., 1994). It may be that a higher rate of endogenous protein secretion, with amino acid infusion, is balanced by a higher rate of protein digestion and absorption, resulting in the similar endogenous amino acid outputs observed in AAI and SAI pigs.

Recent studies (e.g., Grant and Snyder, 1988) indicate that a lack of intestinal and pancreatic fuels is largely responsible for altered gut function, even with total parenteral nutrition. The consumption of a large quantity of starch, in conjunction with amino acid infusion, might counter this, since glucose may also be used as an intestinal fuel. Mucosal mass and nutrient absorption were greater in parenterally fed rats with intralumenal infusion of 30% glucose compared to 10% glucose or saline (Richter et al., 1983). Mucosal enzyme activities, reduced as a result of starvation, are rapidly restored following the consumption of diets containing free amino acids (Poullain et al., 1989). However, in the absence of dietary amino acids in the intestinal lumen the intestinal mucosa looks primarily to the plasma for amino acids for the synthesis of at least some of these enzymes (Dudley et al., 1992). Thus, in addition to providing amino acids for the synthesis of mucosal enzymes, the provision of amino acids parenterally might also prevent the decline in pancreatic protease secretion observed in pigs fed protein-free diets (Corring and Saucier, 1972; Hee et al., 1988).

Mucin represented approximately 5 to 11% of the total endogenous protein in ileal digesta (Table II-4). As expected the contributions of threonine (28 to 35%), serine (13 to 16%) and proline (7 to 24%) were highest. Amino acids from mucin contributed more to amino acids in digesta of AAI pigs than SAI pigs, reflecting both the lower output of endogenous protein (de Lange et al., 1989) and the higher output of mucin in ileal digesta from these pigs. These results support the premise that the presence of mucin in ileal digesta is a primary reason for the low ileal digestibilities of threonine, observed for many feedstuffs (e.g., Sauer and Ozimek, 1986). Degradation of mucin by colonic bacteria (Hoskins, 1984), would explain the large disappearance of these amino acids in the large intestine (Sauer and Ozimek, 1986).

The daily output of total and nonmucin endogenous protein at the distal ileum is presented in Table II-5. The effect of amino acid infusion on the daily output of endogenous protein in these pigs was discussed previously (de Lange et al., 1989). Owing to the relatively small contribution of mucin to endogenous

protein in ileal digesta (Table II-4), the composition of nonmucus endogenous protein was little changed from that of total endogenous protein. However, as expected, the contribution of most amino acids was increased at the expense of threonine, serine and proline, the primary amino acids in mucin. Whereas threonine is typically the largest contributor to indispensable amino acids in endogenous protein (e.g., Sauer et al., 1977; de Lange et al., 1989), its importance in nonmucin endogenous protein is considerably less

With two notable exceptions the composition of nonmucin protein in CM (Table II-5) also resembled that of total endogenous protein in ileal digesta. Firstly, the contributions of the major mucin amino acids, threonine, serine and proline, were higher than their counterparts in ileal digesta, suggesting that the actual content of mucin protein was underestimated. The higher content of these amino acids in mucin subunits suggest that soluble mucin in ileal digesta is predominantly, if not entirely, in the subunit form. The protein content of mucin in this study would need to be 20 to 30% higher than estimated in order to lower the contributions of these amino acids in nonmucin protein in CM to that observed in other endogenous proteins, such as those from the pancreas (Corring and Jung, 1972). While this underestimation will have only a small effect on estimates of mucin output at the distal ileum, given the small content of mucin protein, it will affect estimates of the contribution of protein from mucin to total endogenous protein. The second notable difference between the composition of nonmucin protein in CM and total endogenous protein is the low glycine content of nonmucin protein in CM (Table II-5). A large portion of the glycine in endogenous protein is likely derived from bile (Sambrook, 1981). Because of the limited solubility of bile acids, glycine derived from bile would not be expected to be prevalent in CM preparations.

The validity of these estimates for mucin output relies on the source of the various endogenous carbohydrates, particularly amino sugars, in ileal digesta. With the exception of glucose and xylose, mucin was estimated to account for most of the carbohydrates, approximately 75% of the fucose and galactose and

53% of the GlcNAc, in ileal digesta (Table II-4). The high content of glucose in ileal digesta (Table II-6) is derived predominantly from the dietary cellulose which is poorly digested in the small intestine (Englyst and Cummings, 1985, 1986). Glucose is a relatively minor component of endogenous carbohydrates in canine Heidenhain pouch secretions (Kowalewski et al., 1976) and in ileal digesta from humans fed a polysaccharide-free diet (Englyst and Cummings, 1985, 1986). Xylose is also probably derived from the diet, since it is not detected in ileal effluent of humans fed polysaccharide-free diets (Englyst and Cummings, 1985, 1986). Although the carbohydrate content of the diet was not determined in this study, xylose was present in a purified diet containing a similar content of starch in a previous study (Nyman and Asp, 1982). Similarily the arabinose and at least some of the mannose and galactose would be derived from the diet.

Potential sources of nonmucus amino sugars, which might interfere with estimates of mucin output, include bacteria, proteoglycans, glycolipids and glycoproteins derived from other sources, including saliva, the pancreas, the intestinal epithelium and bile. Amino sugars derived from bacterial cells are unlikely to contribute substantially to amino sugars in ileal digesta. Rhamnose and/or ribose contents in the predominant strains of bacteria in ileal digesta of cannulated pigs, *Streptococcus faecalis* and *Escherichia coli* (Dierick et al., 1986), are as important as that of the amino sugars (Walla et al., 1984; Gilbart et al., 1988), however, these sugars were only present in trace amounts in ileal digesta in this study. This is consistant with observations in feces in which the maximum contribution of ribose and rhamnose from bacteria to fecal solids is at most 0.7% despite the fact that bacteria represent more than 50% of fecal solids (Stephen and Cummings, 1980). The muramic acid, GlcNAc and GalNAc contents of feces from humans fed a low-fiber diet were 0.2, 0.7 and 0.2%, respectively, representing only 0.06, 0.21 and 0.06 g/day (Kraus et al., 1990). The presence of proteoglycans in ileal digesta could in part account for the relatively low contribution (47 to 58%) of mucin to total GlcNAc in ileal digesta since uronic acids and GlcNAc constitute essentially all of the carbohydrate in proteoglycans (Roden and Horowitz, 1978).

Although uronic acids were not determined in ileal digesta in this study, substantial quantities were observed in ileal effluent from humans fed polysaccharide-free diets (Englyst and Cummings, 1986).

The presence of glycoproteins and glycolipids is also of interest since, with the exception of high glucose and mannose contents, their composition somewhat resembles that of mucin, although the GalNAc content is low (Kim et al., 1984). However, despite their prevalence in membrane glycoproteins, mannose and glucose are only minor constituents of endogenous carbohydrates in ileal effluent (Englyst and Cummings, 1986). The importance of membrane glycoproteins in CM is unclear since glucose and mannose are commonly found in purified mucin preparations (Mantle and Allen, 1981; Snary and Allen, 1971). Overall, given the potential recovery of dietary carbohydrates in ileal digesta, the contribution of nonmucin carbohydrates to total carbohydrates is likely to be relatively low. This is particularily true for GalNAc which is a relatively minor component of nonmucin amino sugar sources. A high content of mucin sugars, in ratios consistant with that of gastric mucin, were also observed in secretions from canine Heidenhain pouches (Kowalewski et al., 1976). Englyst and Cummings (1985, 1986) report that the majority, approximately 65%, of endogenous carbohydrates in ileal effluent of humans consisted of fucose and galactose. More recently, Monsma et al. (1992) reported that mucin carbohydrates, fucose, galactose and the amino sugars, represented 77% of the total carbohydrates in ileal digesta from colectomized rats. These observations are consistant with the results of this study, demonstrating that mucin represents the primary source of endogenous carbohydrate in ileal digesta.

The isolation of CM from ileal digesta has important consequences for nutritional studies. The separation of endogenous protein from undigested dietary protein is a major impediment in the assessment of the effects of dietary treatments on the digestion of protein and the true value of different dietary ingredients. In particular, information on the contribution of different sources of endogenous protein, to total endogenous protein recovered at the distal ileum, is

lacking. Such information would provide valuable insights into the effect of dietary constituents, such as protein and fiber, on the digestive process. In this respect, the CM isolation procedure employed in this study, and a method for estimating mucin output in ileal digesta, is an important step forward to provide such information.

Table II-1. Recovery (g/day) and composition (%) of crude mucin from ileal digesta of pigs fed a protein-free diet while administered either saline (SAI) or a complete amino acid mixture (AAI) intravenously

Item	SAI	AAI	SEM[a]	P[b]
Crude mucin	5.2	5.6	0.13	0.47
Protein[c]	34.3	35.1	2.73	0.79
Fucose	3.5	4.8	0.36	0.21
Mannose	1.2	1.0	0.04	0.11
Glucose	1.6	1.2	0.40	0.09
Galactose	8.2	10.3	0.31	0.12
GlcNAc	8.7	9.7	0.11	0.11
GalNAc	10.7	12.5	0.28	0.14
Prtn/Carb	1.01	0.90	0.085	0.56
GluNAc/GalNAc	0.82	0.78	0.109	0.83

[a]Standard error of the mean.
[b]Probability of difference between SAI and AAI pigs.
[c]Sum of individual amino acids.

Table II-2. Composition (mol/100mol) of carbohydrate and protein in crude mucin from ileal digesta of pigs fed a protein-free diet while administered either saline (SAI) or a complete amino acid mixture (AAI) intravenously

Items	SAI	AAI	SEM[a]	P[b]
Carbohydrates				
Fucose	13.7	15.6	0.56	0.21
Galactose	29.4	30.4	0.25	0.19
GlcNAc	25.4	23.5	0.97	0.34
GalNAc	31.5	30.5	0.16	0.13
Protein				
Threonine	15.0	16.4	0.33	0.17
Proline	12.5	12.4	0.69	0.83
Serine	11.5	12.4	0.74	0.56
Alanine	9.2	9.9	0.63	0.59
Glycine	8.9	8.7	0.65	0.79
Glutamate	8.7	8.2	0.29	0.42
Aspartate	7.8	7.0	0.34	0.33
Valine	5.9	6.0	0.06	0.35
Leucine	5.8	5.2	0.30	0.38
Isoleucine	3.0	2.7	0.07	0.22
Tyrosine	2.6	2.1	0.13	0.23
Arginine	2.5	2.4	0.03	0.19
Lysine	2.5	2.3	0.14	0.50
Phenylalanine	2.4	2.5	0.16	0.44
Histidine	1.3	1.3	0.07	0.47
Methionine	0.7	0.6	0.07	0.70

[a]Standard error of the mean.
[b]Probability of difference between SAI and AAI pigs.

Table II-3. Estimated daily outputs (g) of total, gastric and intestinal mucin in crude mucin and the contribution (%) of carbohydrates and amino acids from mucin to carbohydrates and amino acids in crude mucin[a]

Item	SAI		AAI	
	Subunit[b]	Native[c]	Subunit	Native
Mucin Output				
Total	2.8 ± 0.92	2.9 ± 0.98[d]	3.4 ± 0.73	3.6 ± 0.95
Gastric	0.8 ± 0.37	0.8 ± 0.39	0.8 ± 0.21	0.9 ± 0.22
Intestinal	2.0 ± 0.59	2.1 ± 0.63	2.6 ± 0.54	2.7 ± 0.58
Carbohydrate				
Fucose	123.4 ±12.98		99.6 ± 9.80	
Galactose	131.7 ± 9.30		117.7 ± 8.97	
GlcNAc	99.5 ± 0.67		99.8 ± 0.61	
GalNAc	99.4 ± 0.32		99.8 ± 0.31	
Protein				
Threonine	54.7 ± 3.85	56.5 ± 4.18	55.7 ± 0.90	57.7 ± 0.98
Proline	39.6 ± 2.84	42.0 ± 3.03	44.9 ± 4.79	47.7 ± 5.11
Serine	34.7 ± 2.44	34.5 ± 2.02	34.8 ± 2.38	34.9 ± 2.21
Alanine	6.5 ± 0.73	14.8 ± 1.18	6.4 ± 0.63	15.1 ± 1.25
Glycine	13.7 ± 0.68	21.8 ± 1.25	15.6 ± 0.92	24.6 ± 1.53
Glutamic acid	9.0 ± 0.59	19.6 ± 1.19	10.4 ± 1.16	22.7 ± 2.47
Aspartic acid	5.1 ± 0.26	20.3 ± 0.96	6.3 ± 0.68	25.2 ± 2.57
Valine	25.0 ± 1.90	39.7 ± 3.05	27.5 ± 1.86	43.6 ± 3.01
Leucine	8.5 ± 0.67	24.2 ± 1.73	10.5 ± 1.21	30.4 ± 3.16
Isoleucine	19.6 ± 1.40	36.8 ± 2.60	23.7 ± 2.10	44.6 ± 3.92
Tyrosine	9.7 ± 0.64	21.0 ± 1.38	13.2 ± 1.83	28.7 ± 3.97
Lysine	11.4 ± 1.25	35.3 ± 3.69	13.5 ± 1.53	42.0 ± 4.56
Arginine	11.6 ± 0.58	33.3 ± 2.07	13.7 ± 0.96	38.4 ± 3.26
Phenylalanine	13.3 ± 2.41	29.3 ± 5.34	13.4 ± 1.55	29.6 ± 3.42
Histidine	16.7 ± 2.13	38.5 ± 4.81	17.5 ± 1.65	40.6 ± 3.68

[a]Total, gastric and intestinal mucin estimated from the GlcNAc/GalNAc ratio and the daily output of GalNAc in crude mucin (see text).
[b]Assuming mucin is completely in its subunit form, i.e., complete proteolytic degradation of the naked region.
[c]Assuming mucin is completely in its native form
[d]Mean ± SEM (n = 3).

Table II-4. Estimated daily outputs (g) of total, gastric and intestinal mucin in ileal digesta and the contribution (%) of carbohydrates and amino acids from mucin to carbohydrates and amino acids in ileal digesta[a]

Item	SAI		AAI	
	Subunit[b]	Native[c]	Subunit	Native
Mucin Output				
Total	5.3 ± 1.08	5.6 ± 1.14[b]	5.7 ± 0.80	6.0 ± 0.85
Gastric	1.5 ± 0.51	1.6 ± 0.53	1.4 ± 0.22	1.4 ± 0.23
Intestinal	3.8 ± 0.75	4.1 ± 0.80	4.3 ± 0.65	4.6 ± 0.69
Carbohydrate				
Fucose	73.3 ± 2.60		74.3 ± 3.76	
Galactose	71.3 ± 0.34		82.0 ± 7.67	
GlcNAc	47.2 ± 3.10		58.2 ± 3.00	
GalNAc	99.8 ± 0.09		100.0 ± 0.13	
Protein				
Threonine	28.0 ± 2.68	29.0 ± 2.93	33.2 ± 1.48	34.4 ± 1.61
Proline	7.2 ± 3.42	7.7 ± 3.64	22.4 ± 1.09	23.8 ± 1.14
Serine	13.3 ± 0.64	13.2 ± 0.67	16.3 ± 0.50	16.3 ± 0.51
Alanine	1.6 ± 0.08	3.7 ± 0.16	1.9 ± 0.07	4.6 ± 0.13
Glycine	1.9 ± 0.48	3.0 ± 0.77	2.8 ± 0.37	4.4 ± 0.59
Glutamic acid	1.9 ± 0.11	4.2 ± 0.23	2.4 ± 0.11	5.2 ± 0.25
Aspartic acid	1.1 ± 0.07	4.2 ± 0.32	1.4 ± 0.06	5.5 ± 0.26
Valine	6.5 ± 0.42	10.4 ± 0.64	7.1 ± 0.39	11.3 ± 0.62
Leucine	2.0 ± 0.10	5.8 ± 0.38	2.4 ± 0.14	6.9 ± 0.40
Isoleucine	3.9 ± 0.19	7.3 ± 0.37	4.7 ± 0.33	8.9 ± 0.63
Tyrosine	3.1 ± 0.26	6.8 ± 0.56	3.8 ± 0.37	8.2 ± 0.80
Lysine	1.8 ± 0.03	5.6 ± 0.15	2.1 ± 0.08	6.7 ± 0.22
Arginine	2.4 ± 0.39	6.9 ± 1.07	3.5 ± 0.23	9.7 ± 0.61
Phenylalanine	2.4 ± 0.19	5.3 ± 0.41	2.5 ± 0.18	5.5 ± 0.39
Histidine	3.9 ± 0.26	9.0 ± 0.64	5.0 ± 0.27	11.6 ± 0.63

[a]Total, gastric and intestinal mucin estimated from the GlcNAc/GalNAc ratio in crude mucin and the daily output of GalNAc in ileal digesta (see text).
[b]Assuming mucin is completely in its subunit form, i.e., complete proteolytic degradation of the naked region.
[c]Assuming mucin is completely in its native form
[d]Mean ± SEM (n = 3).

Table II-5. Output (g/day) and composition (%) of total and nonmucin endogenous protein in ileal digesta and nonmucin protein in crude mucin from pigs fed a protein-free diet while administered either saline (SAI) or a complete amino acid mixture (AAI) intravenously

	Endogenous Protein						Crude Mucin					
	Total			Nonmucin[a]			Subunit[b]			Native[c]		
Item	SAI	AAI	SEM	SAI	AAI	SEM	SAI	AAI	SEM	SAI	AAI	SEM
Protein[d]	15.1	11.0	1.30	14.3	10.2	1.30	1.3	1.5	0.07	1.1	1.3	0.08
Indispensable Amino Acids												
Arginine	3.9	4.1	0.77	4.0	4.3	0.09	4.2	4.1	0.05	3.6	3.4	0.02
Histidine	1.5	1.8	0.10	1.6	1.8	0.11	1.8	1.9	0.09	1.5	1.6	0.14
Isoleucine	3.6	4.4	0.31	3.7	4.6	0.33	3.4	3.1	0.05	3.1	2.6	0.11
Leucine	5.9	7.4	0.45	6.1	7.8	0.48	7.7	7.0	0.23	7.2	6.4	0.36
Lysine	4.3	5.2	0.19	4.5	5.5	0.21	3.6	3.3	0.13	3.0	2.6	0.23
Phenylalanine	3.8	5.5	0.29	3.9	5.8	0.31	3.7	4.1	0.19	3.5	3.9	0.25
Threonine	6.4	8.1	0.46	4.9	5.9	0.32	8.9	10.7	0.17	9.7	11.1	0.06
Valine	4.9	6.7	0.51	4.8	6.7	0.52	5.7	5.8	0.05	5.2	5.2	0.13
Dispensable Amino Acids												
Alanine	6.1	7.2	0.40	6.3	7.6	0.43	8.5	9.5	0.88	8.8	10.0	1.05
Aspartic Acid	9.3	10.6	0.53	9.7	11.3	0.58	10.8	10.1	0.19	10.3	9.3	0.30
Glutamic Acid	10.9	12.9	0.84	11.3	13.6	0.91	12.7	12.3	0.11	12.8	12.4	0.10
Glycine	9.3	9.2	0.04	9.7	9.7	0.00	6.3	6.3	0.68	6.5	6.6	0.81
Proline	21.7	7.0	4.87	21.6	5.9	5.03	9.6	9.0	1.09	10.4	9.9	1.11
Serine	5.8	6.9	0.44	5.3	6.2	0.41	8.7	9.7	0.85	9.9	11.3	1.12
Tyrosine	2.6	3.2	0.29	2.7	3.4	0.31	4.6	3.8	0.16	4.6	3.6	0.19

[a]Daily output and composition of endogenous protein corrected for the output of mucin protein assuming mucin in ileal digesta is completely proteolytically degraded.
[b]Output and composition of nonmucin protein in crude mucin assuming all soluble mucin is proteolytically degraded.
[c]Output and composition of nonmucin protein in crude mucin assuming all soluble mucin is in its native form.

Table II-6. Ileal output (g/day) of carbohydrates from pigs fed a protein-free diet while administered either saline (SAI) or a complete amino acid mixture (AAI) intravenously[a]

Item	SAI	AAI	SEM[b]	P[c]
GalNAc	1.07	1.16	0.003	0.0533
GlcNAc	.89	1.55	0.021	0.0505
Fucose	[illegible]	0.58	0.008	0.2792
Galactose	1.50	1.37	0.038	0.2520
Glucose	41.24	37.74	1.005	0.2559
Mannose	0.96	0.67	0.005	0.0159
Xylose	7.44	7.46	0.384	0.9147

[a]Traces of arabinose, rhamnose and ribose were also detected.
[b]Standard error of the mean.
[c]Probability of difference between [illegible]d AAI pigs.

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CHAPTER III

NUTRIENT DIGESTIBILITIES AND EFFLUENT MUCIN OUTPUT IN HUMAN ILEOSTOMATES CONSUMING FIBER SUPPLEMENTED TOTAL ENTERAL NUTRITION FORMULAS

A. Introduction

Dietary fiber supplementation of total enteral nutrition (TEN) formulas is proposed to have many benefits, including the maintenance of small intestinal and large bowel function (Silk, 1989; Scheppach et al., 1990). However, nutrient losses from the small intestine have been reported to increase with the ingestion of dietary fiber (Jenkins et al., 1987; Steinhart et al., 1992; Ellegard and Bosaeus, 1991; Zhang et al., 1991, 1992, 1994) which may be detrimental, especially in critically-ill patients who may not be meeting their nutrient requirements.

Human ileostomy studies offer a direct and quantitatively accurate approach to determine the effect of dietary components on small bowel excretion (Zhang et al., 1994; Sandberg et al., 1981, 1986; Englyst and Cummings, 1985; Chapman et al., 1985). The ileostomate is an ideal model for studies assessing the effects of dietary fiber on small intestinal nutrient digestion and absorption since much of the digestion that occurs in the large intestine is the result of microbial fermentation (Wrong, 1988; Cummings and Englyst, 1987). The provision of fermentable energy sources such as dietary fiber stimulate microbial growth and the *de novo* synthesis of carbohydrates and protein, increasing their excretion in feces (Cummings et al., 1979; Stephen and Cummings, 1979; Chapman et al., 1985). Because of the bacterial synthesis of amino acids, proteins and carbohydrates in the large bowel and subsequent excretion in feces,

digestibility studies assessing ileal effluent are more sensitive to dietary manipulations than are those using fecal samples (Sauer and Ozimek, 1986).

This study was undertaken to determine the effect of soy fiber (SF) supplementation of TEN formulas on the absorption of protein and carbohydrates in the small intestine of human ileostomates. The effect of SF consumption on the ileal output of gastrointestinal mucin was also studied.

B. Experimental Procedures

Subjects

Eight healthy ileostomates, four men and four women, with a mean age of 52 years, volunteered for this study (Table III-1). Subjects were not taking any medication during the study and refrained from caffeinated and alcoholic beverages throughout the collection periods. All participants had had colectomies for ulcerative colitis. Ileostomies had been in place for an average of 6 years. Mean Body Mass Index (BMI) was 24.67±1.72 kg/m^2 (range 17.40 to 30.47 kg/m^2). Subjects gave their informed consent to participate in this study, which was approved by the Ethics Review Committee for Human Experimentation, Faculty of Medicine, University of Alberta.

Diets

Energy and nutrient requirements were derived from 7 day diet histories, activity levels and recommended nutrient requirements for the individuals. Participants were then randomized to one of five TEN diets to meet energy requirements. Subjects consumed each of the test diets in random order with a minimum 7 day washout period between tests. Each test diet was consumed for three consecutive days with the intake divided over 6 equal portions consumed at 0800, 1000, 1200, 1400, 1600 and 1800 daily. Aman et al. (1994) did not find differences in the excretion of dietary fiber or dietary fiber components in ileal

effluent among the 2, 3 or 7 d on test diets. Similarly, we did not observe any differences ($P > 0.05$) between digestibility measurements between d 1 and 2 in this study. The diets consisted of Ensure (Ross Laboratories, Columbus, OH) and Enrich (Ross Laboratories) in the following ratios 1:0, 0.75:0.25, 0.50:0.50, 0.25:0.75 and 0:1. These were designated as Tests 1, 2, 3, 4 and 5, respectively. The females had lower energy requirements and therefore consumed less TEN, but there were no other differences with regards to weight or BMI (Table III-1). Because each person consumed a sufficient volume of TEN to meet his/her energy needs, and because test diets contained increasing levels of SF, 1.0 to 16.5 g/L for diets consisting of Ensure only (Test 1) to those consisting of Enrich only (Test 5), a unique continuum of SF intakes, from 3.3 to 38.9 g/d, was established.

Protocol

Ileostomates fasted from 2000 on the d prior to the initiation of each part of the study. At 0800 on test d 1, prior to consumption of the first portion, ileostomy bags were drained and the contents discarded. Subjects were requested to empty appliances frequently into plastic containers, which were to be frozen immediately at -20° C, during the collection periods. The overnight collection was taken at 0800 on the following d and the procedure repeated for a second consecutive d (test d 2). Effluent was pooled within individual and test day, weighed wet, freeze-dried and weighed again prior to further analysis. For the three menstruating females, the test d were confined to the follicular phase to minimize changes in transit rate associated with the menstrual cycle (McBurney, 1991). The average plasma progesterone level during the tests was 4.4 nmol/L (range 0.5 to 27.1). Progesterone levels were elevated in two individuals for one Test each (15.7 and 27.1 nmol/L; range without these values was 0.5 to 5.7) but this did not influence the results of those tests.

Chemical analyses

Dry matter and N were determined according to AOAC (1988) procedures.

Crude mucin analyses. Crude mucin (CM) was isolated as described in Chapter II. Approximately 1 g of freeze-dried ileal effleunt was combined with 25mL 0.15M sodium chloride, containing 0.02M sodium azide at 4°C. Samples were homogenized for 1 min using a Polytron homogenizer (Kinematica, Kriens, Switzerland) and immediately centrifuged at 12,000 x g for 30 min. The supernatant was decanted into a second test tube and centrifuged once again. Fifteen mL of the aqueous fraction was pipetted into a preweighed 50 mL test tube and the samples cooled in an ice-bath. Ice cold ethanol was added to a final concentration of 60% (v/v) and the samples were allowed to precipitate overnight at -20°C. Crude mucin was recovered by centrifugation at 1400 x g for 10 min. Samples were rinsed by redissolving the precipitate in 0.15M NaCl, cooling in an ice bath, and precipitating with ice cold ethanol. Samples were rinsed until a clear supernatant was achieved (twice). The CM was solubilized in 10 mL distilled water, frozen and freeze-dried.

Amino acid analysis. Approximately 50 mg of freeze-dried enteral formula or ileal effluent, or 30 mg of CM, was weighed into 10 mL screw cap test tubes. Six mL of 6M HCl was added, the tubes purged with nitrogen and the samples hydrolyzed at 110°C for 24 h. Following hydolysis, 1 mL of water and 200 µL of internal standard were added. Internal standards contained both ß-amino-n-butyric acid and ethanolamine (Sigma Chemical Co., St. Louis, MO) at 25 µmol/mL in water. Amino acid standards were prepared by combining 1 mL of Sigma amino acid standard (2.5 µmol/mL), 200 µL internal standard and 6 mL 6M HCl in a screw cap test tube. Samples were prepared for, and analyzed by high performance liquid chromatography, according to procedures described by Jones and Gilligan (1983). Anlysis was carried out using a Varian Vista 5500 Liquid Chromatograph (Varian instruments, Walnut Creek, CA).

Sugar analysis. The contents of neutral and amino sugars in samples were determined according to procedures described previously (Chapter II). Briefly, 50 mg of enteral nutrition formula, ileal effluent or CM were incubated in 3 mL of 12M sulfuric acid for 1 h at room temperature. The samples were diluted to

3M with the addition of water, purged with nitrogen, and hydrolyzed at 110°C for 1 h. Following hydrolysis, 200 µL of internal standard, N-methylglucamine and *myo*-inositol (Sigma) at 10 mg/mL for amino and neutral sugars, respectively, were added. Aliquots (1 mL) of the hydrolysate were made basic with the addition of 0.7 µL of concentrated NH_4OH. Sugars were reduced by adding 100 µL of the basic samples to 1 mL of sodium borohydride (30 mg/mL in dimethylsulphoxide; Sigma) and heating at 40°C for 90 min. Following the decomposition of excess sodium borohydride with glacial acetic acid (0.2 mL), 1-methylimidazole (0.2 mL) and then acetic anhydride (2 mL) were added and acetylation allowed to proceed for 10 min. Acetylation was stopped with the addition of 5 mL of water and the mixture cooled to room temperature. Alditol acetates were extracted into 4 mL of dichloromethane, rinsed twice with water (5 mL) and evaporated to dryness under a stream of nitrogen. Samples were redisolved in 1 mL dichloromethane prior to analysis on a DB-17 capillary column (0.25 mm i.d. x 30 m; J&W Scientific, Folsom, CA) in a Varian 3400 gas-liquid chromatograph. Instrument conditions have been described in detail (Chapter II).

Calculations and Statistical analyses

Differences between dietary treatments (Tests) and level of intake were determined using a randomized block design with individuals and test d as sources of variation. The individual within intake level was used as the source of error for level of intake and the individual x test diet, within level of intake, was used as the source of error for dietary treatment and the interaction between dietary treatment and level of intake (Steel and Torrie, 1980). Significant treatment means were compared with the aid of the Student-Newman-Keuls multiple range test. Analyses were performed using SAS (1990) general linear models procedures. Regression analyses were performed using SAS (1990) regression procedures according to principles outlined by Steel and Torrie (1980). Significance of differences between sexes for intercept and slope values for regressions of component outputs in ileal effluent or CM versus soy fiber intake

were determined using the following regression equation:

$$Y = \alpha_0 + \alpha_1 X + \alpha_2 + \alpha_2 X$$

where Y = daily output of a component in effluent or CM and X = daily consumption of soy fiber, α_2 is 1 or -1 for females or males, respectively, and measures the effect of sex on the intercept. The effect of sex on the slope of the regressions is determined by $\alpha_2 X$. Where significant differences were observed between sexes for either the intercept or the slope, regressions were performed separately by sex.

C. Results

The composition of the enteral nutrition formulas is presented in Table III-2. The amino acid contents of Ensure and Enrich were 3.3 and 3.4 g/100mL, respectively, accounting for most (90%) of the protein. Soy fiber contents of the enteral formulas, 1.0 and 16.5 g/L for Ensure and Enrich, respectively, were those determined by Fredstrom et al. (1991). The analyzed carbohydrate contents, 10.5 and 11.7 g/100mL for Ensure and Enrich, respectively, were somewhat lower than the label claim for these products, 14.5 and 16.3g/100mL, respectively. However, our analysis suggests, based on the mannose content, that the formulas contained about 1 g sucrose/100 mL compared to values of 5.6 and 5.2 g/100 mL reported by Peters and Davidson, (1992) for Ensure and Enrich, respectively, indicating that the low carbohydrate content was the result of an underestimation in the sucrose content. Correcting for this discrepancy gives a carbohydrate content approximating that claimed on the label. Fructose could not be separated from mannose under the conditions of this study.

Daily outputs of water, DM, neutral carbohydrate and N in ileal effluent versus SF intake is presented in Figure III-1. Effluent output (overall mean 533±21.5 g/d), which exhibited similar patterns to that of water output (493±20.6 g/d), are not presented. Effluent and effluent water outputs were not different

between males and females and increased ($P < 0.01$) with increasing SF intake. Effluent DM and neutral carbohydrates increased linearly ($P_{slope} < 0.0001$) with increasing SF consumption but effluent N was unaffected. Intercept values for DM and N outputs were nearly twice as high in males compared to females, 33.2±2.87 versus 18.6±1.91 g/d and 1.8±0.01 versus 1.0±0.01 g/d, respectively. Although not significant, neutral carbohydrate output was also higher in males, 5.5±1.36 versus 2.5±0.78 g/d.

Individual effluent carbohydrates versus SF intake are presented in Figure 2. Neither the intercepts nor the slopes for galactose, glucose, arabinose or xylose were different ($P > 0.05$) between males and females. No endogenous arabinose, xylose or ribose (range 0 to 1.1 g/d; not presented) was detected ($P_{intercept} > 0.10$). Intercept values for the remaining carbohydrates were at least twice as high in males. All effluent neutral carbohydrates increased linearly ($P_{slope} < 0.005$) with increasing SF intake, however, amino sugar outputs were unaffected ($P_{slope} > 0.10$).

The recovery of DM and carbohydrates in ileal effluent following the consumption of the different Test diets are presented in Table III-3. Recoveries of DM and total neutral carbohydrates, as well as mannose and glucose, increased as the proportion of Enrich was increased in the diet. Recoveries of fucose and galactose declined, while those of arabinose and xylose were unaffected by increasing the consumption of Enrich. Glucose digestibility for Ensure was 98.4±0.18% compared to 95.6±0.17% for Enrich. Recoveries for arabinose, xylose and galactose were approximately 100% for Tests 3, 4 and 5 indicating that little digestion of SF NSP occurred in the small intestine.

Nitrogen and amino acid digestibilities were not influenced ($P > 0.30$) by the proportion of Enrich consumed, therefore, only the mean digestibilities are presented (Table III-4). Apparent digestibilities for N and all amino acids tended to be higher ($P < 0.15$) for females, therefore, digestibilities are presented separately for each sex. With the exception of threonine, apparent digestibilities of N and the indispensable amino acids were greater than 85%. Apparent digestibilities of the dispensable amino acids ranged from 65% for glycine to 90%

for glutamic acid. True N digestibility was approximately 8 percentage units higher than the apparent value, while true amino acid digestibilities were 3 (glutamic acid) to 18 (glycine) percentage units higher than apparent values.

The daily output of CM and carbohydrates in CM increased linearly (P_{slope} < 0.0001) with increasing consumption of SF (Figure III-3). The daily output of protein (sum of the amino acids) in CM was unaffected (P_{slope} > 0.10). The amino acid composition of CM was largely unaffected by the consumption of the different Test diets, although contributions from aspartic acid and threonine did tend to increase at the expense of serine as the proportion of Enrich in the diet was increased (Table III-5). Serine and threonine were important contributors to CM protein. Mucin carbohydrates, galactose, fucose, N-acetylglucosamine (GlcNAc) and N-acetylgalactosamine(GalNAc) accounted for most (85.5%) of the carbohydrate content of CM from subjects consuming the low-fiber diet. With the exception of galactose, the contributions of these sugars declined in conjunction with SF intake.

Arabinose, fucose, galactose, ribose and xylose in CM increased linearly (P_{slope} < 0.001) with increasing SF (Figure III-4), while glucose, mannose and the amino sugars were unaffected (P_{slope} > 0.05). The intercepts of arabinose, ribose and xylose were not different from zero (P > 0.10), whereas those of mucin carbohydrates, galactose, fucose and the amino sugars, as well as glucose and mannose were (P < 0.001). Similar to effluent carbohydrates, intercept values for males were up to twice that in females. The carbohydrate composition of mucin (mol/100mol), determined by extrapolation to zero SF intake, 40.9% galactose, 30.0% GluNAc, 19.5% fucose, and 9.6% GalNAc, was similar in both sexes. Glucose and mannose are commonly associated with mucin preparations (Allen, 1981).

Endogenous carbohydrates in ileal effluent, determined by extrapolation to zero SF fiber intake are presented in Table III-6. Mucin carbohydrates in ileal effluent, estimated from the composition of mucin carbohydrates in CM and the content of GalNAc in effluent (Chapter II), are also presented. Next to glucose,

galactose and GlcNAc were the highest contributors to endogenous carbohydrates. Endogenous carbohydrates were approximately twice as high in males compared to females. With the exception of glucose and mannose, mucin accounted for approximately 90% of endogenous carbohydrates. Effluent amino sugars increased linearly ($P < 0.01$), in both males and females with increasing effluent DM (Figure III-5).

D. Discussion

The most pronounced effect of adding SF to enteral diets is its influence on the recovery of DM in ileal effluent. The complete recovery of SF in ileal effluent essentially accounted for all of the increase in DM output, approximately 1.0 g effluent DM/g SF consumed. This was also expressed by the complete recovery of NSP carbohydrates since neutral carbohydrate output also increased at a similar rate to soy fiber consumption, 0.83 g/g SF fiber consumed. Uronic acid, representing up to 20% of SF (Shinnick et al., 1989; Titgemeyer et al., 1991), is expected to make up the difference between carbohydrate and DM outputs. That arabinose and xylose from SF were not digested in the small intestine is reflected in the fact that their recoveries were not influenced by the proportion of Enrich in the diet and that they were approximately 100%. Arabinose and Xylose were not present in ileal effluent of humans fed NSP-free diets (Englyst and Cummings, 1985, 1986, 1987). The decline in fucose and galactose recoveries with increasing consumption of Ensure is the result of the dilution of endogenous carbohydrates by SF NSP.

The complete recovery of SF NSP is consistant with the results of several recent studies, particularily those of Englyst and Cummings (1985, 1986, 1987) and Sandberg et al. (1981), which have demonstrated that NSP from a variety of dietary sources are not digested in the small intestine. Jenkins et al. (1987) recorded a mean ileal recovery of fiber of 91.5% from 20 different foods given to

an ileostomates. While the recovery of NSP at the distal ileum indicates that SF fiber has little direct nutritional value in the small intestine, the added bulk has important physiological consequences. Most notable of these is its potential role in preventing ileal mucosal atrophy associated with feeding fiber-free enteral diets (Silk, 1989). In addition, carbohydrate entering the large intestine is available for bacterial fermentation, producing short-chain fatty acids which have important physiological responses and contribute to overall energy requirements (McNeil, 1984; Cummings and Englyst, 1987; McBurney, 1991).

Despite large variations, an increase in daily effluent and effluent water outputs was observed with increasing SF consumption, approximately 7.7 and 6.5 g/g SF intake, respectively. Several studies (Chacko and Cummings, 1988; Ellegard and Bosaeus, 1991; Zhang et al., 1992) have reported increases in total effluent outputs with fiber consumption which were not completely accounted for by the increase in effluent dry matter. Effluent volumes were unaffected, although they tended to increase, with increasing consumption of dietary fiber from different foods in a recent study by Steinhart et al. (1992). The higher daily output of effluent in this study is related to the recovery of SF in ileal effluent, directly via its influence on DM output and indirectly via its water holding capacity (Vahouny, 1987; Kritchevsky, 1988).

Absorption of the 100 to 200 g of starch in the enteral formulas was almost complete for Ensure, 98.4%, similar to that observed by Chapman et al. (1985). This value most likely reflects some malabsorption of starch since endogenous glucose output, 1.6 and 2.6 g/d in males and females, respectively (Table III-6), was considerably higher than that observed in ileal effluent following the consumption of NSP-free diets, approximately 0.4 g/d (Englyst and Cummings 1985, 1986, 1987). The lower digestibility of glucose for Enrich, 95.6%, reflects the recovery of NSP glucose from SF in ileal effluent, however, it may also include some effect of SF consumption on starch absorption. Jenkins et al. (1987) have suggested that fiber intake may be the primary determinant of the amount of available carbohydrate entering the large intestine. A recent study by Steinhart

et al. (1992) demonstrated that the loss of available carbohydrate in ileostomy effluent was correlated with dietary fiber intake. However, the consumption of Enrich has little effect on serum glucose response when compared to Ensure (Shinnick et al., 1989; Peters and Davidson, 1992) indicating, as suggested by the results of this study, that the addition of SF fiber to enteral nutrition formulas has little detrimental effect on the utilization of starch.

Effluent N and amino acid outputs were unaltered with increasing consumption of SF (Figure III-1). These results are in agreement with those of Sandberg et al. (1981) in which effluent nitrogen output was unaffected by the consumption of 16 g of wheat bran. Although, significant increases in the output of N in ileostomy effluent have been observed following the consumption of some insoluble fibers (Ellegard and Bosaeus, 1991; Zhang et al., 1991, 1992, 1994), the excess N output appears to be derived from N associated with the fiber matrix. On the other hand, soluble fibers, such as guar gum (Higham and Read, 1992) and pectin (Sandberg et al., 1983), have been shown to decrease ileal protein digestibility. The difference in these observations is attributed primarily to the viscous nature of guar gum and pectin and its negative affects on enzyme activity and nutrient absorption (Vahouny, 1987; Kritchevsky, 1988).

Daily N and amino acid outputs were higher in males than in females (Figure III-1). Although this may in part be related to the consumption of a larger volume of TEN formula by males, N and amino acid digestibilities, particularily those in females where the largest differences in TEN volume consumed were present, indicate that there is a real basis for a difference in these outputs between sexes. Average N digestibilities in females consuming approximately 1 L TEN/d (1155 and 1174 kcal/d) were similar to that of females consuming 1.6 L TEN/d (1744 kcal/d), 87.0 and 86.6%, respectively. However, these values were approximately 3 percentage units higher than those in males consuming 1.8 (1993 kcal/d) and 2 L TEN/d (2250 kcal/d), 83.2 and 84.1%, repectively, indicating a proportionally higher output of N in males. Similar results were obtained for amino acids. The higher relative output of N and amino acids in males accounts

for the lower digestibilities observed in these subjects (Table III-4). This is likely due to a higher output of endogenous protein in ileal effluent, as opposed to a lesser digestion of TEN protein, since daily outputs of endogenous carbohydrates and mucin were also higher in males.

The apparent N digestibilities for both Ensure and Enrich were approximately 85%. The apparent ileal digestibilities for most amino acids were greater than 85%, although several amino acids, particularily aspartate (77.1±0.61%), glycine (68.5±0.84%), and threonine (78.6±0.53%) had lower digestibilities. These amino acids are important constituents of endogenous protein (Rowan et al., 1993; Fuller et al., 1994) and their low digestibilities suggest that a considerable amount of the protein and amino acids in ileal effluent is endogenous in origin.

True ileal N and amino acid digestibilities were calculated using daily endogenous outputs reported by Fuller et al. (1994). Daily endogenous outputs, rather than outputs on a DM intake basis (Rowan et al., 1993), were used because Furuya and Kaji (1992) have recently provided good argument to suggest that endogenous nitrogen and amino acid outputs are constant on a daily basis, relatively unaffected by dietary fiber and DM intakes, which is supported by observations within sex in this study. True digestibilities for the TEN formulas, 93.2 and 92.0% for N and total amino acids, respectively, were only moderately lower than that reported for casein, approximately 97% overall, in pigs (Furuya et al., 1986; Furuya and Kaji, 1989). Pigs are a suitable model for studying protein digestion in humans (Rowan et al., 1994). Casein, with a lesser contribution from soy protein, is the major source of protein in the TEN formulas consumed in this study. The difference between these values might reflect the presence of soy protein isolate, which has been shown to depress protein digestibilities in diets containing meat protein (Sandstrom et al., 1986). Alternatively, the difference may simply result from the more direct method of determining true digestibilities in the previous studies since Young and coworkers (Scrimshaw et al., 1983; Wayler et al., 1983; Young et al., 1984; Istfan et al., 1983), through a series of N

balance studies, demonstrated that soy protein isolates were equivalent in quality to casein, meat and egg.

The final objective of this study was to determine the effect of the addition of soy fiber to TEN formulas on the dail output of mucin in ileostomy effluent. Similar to a previous study with pigs (Chapter II), CM recovered from ileal effluent contained predominantly those sugars associated with mucin, demonstrating a high content of this glycoprotein. Galactose, fucose, GlcNAc and GalNAc accounted for more than 85% of CM carbohydrates in subjects consuming the low-fiber diet. Crude mucin, however, also contained a fraction of carbohydrates which were derived from dietary NSP, the contribution of which increased in proportion to their content in the diet. Soluble fractions of dietary fiber also precipitate in cold ethanol (Englyst and Cummings, 1988). Also similar to our previous study (Chapter II), there was a high content of nonmucus protein. Following extensive dialysis and gel-permeation chromatography most of these components remain associated with mucin (Clamp and Gough, 1991). Removal of these contaminants is a long and laborious procedure (Allen, 1981) that is not feasible for a large number of samples. However, useful information concerning the output and origin of mucin in ileal effluent can be obtained from the composition of CM and the daily output of amino sugars (Chapter II).

The composition of CM was similar in all subjects except one. This individual, a female, who had a higher content of GalNAc with a GlcNAc/GalNAc ratio in CM of 1.4±0.01 compared to 3.0±0.07 in the remaining subjects. A low ratio was also observed in effluent, 1.6±0.02 versus 3.5±0.07. This high content of GalNAc is indicative of an A secretor which, unlike B, H or nonsecretors, have a terminal GalNAc residue on mucin oligosaccharide chains (Allen, 1981; Neutra and Forstner, 1987; Mantle and Allen, 1989). N-Acetylgalactosamine residues occur only at either end of the oligosaccharide chains. Mantle et al. (1984) reported a GlcNAc/GalNAc ratio of 0.9 in small intestinal mucin from a patient with blood group A compared to 2.2 for a patient with blood group H. The GlcNAc/GalNAc ratio in human gastric mucus from

subjects with blood type A is approximately 1.8 compared to 2.7 and 2.4 for subjects with blood types O and B, respectively (Schrager and Oats, 1974).

Overall, the high GlcNAc/GalNAc ratio in CM, in conjunction with its high serine content, suggests that mucin in ileal effluent was predominantly of gastric origin (Mantle and Allen, 1989). The GlcNAc/GalNAc ratios in CM from individuals in this study, excluding the one individual, ranged from 2.5 to 4.1 (mean 3.0±0.07) which is roughly in the range of that reported for human gastric mucin (Schrager and Oats, 1974). A high proportion of gastric mucin in ileal effluent is not surprising since gastric mucus secretion is apparently governed by many of the same neural and hormonal controls regulating digestive processes (Mantle and Allen, 1989). More importantly, pepsin secretion is similarly regulated and its degradation of mucus gels is well documented (Allen, 1981; Mantle and Allen, 1989). Relative increases of mucin carbohydrates in the lumen of canine Heidenhain pouches were similar to that of pepsin following stimulation with food, secretin and histamine (Kowalewski et al., 1976). The consumption of food stimulated a 44% increase in the amount of mucin carbohydrates and a 300% increase in pepsin. In addition, as a result of the high osmolarity of TEN formulas, gastric emptying is delayed (Paraskevopoulos et al., 1988), allowing pepsin to remain in contact with the gastric mucus layer for a longer period of time.

A greater proportion of gastric mucin is in contrast to observations in the previous Chapter (II) in which the mucin in ileal digesta of pigs fed a protein-free diet originated predominatly from the small intestine. The higher proportion of intestinal mucin in the previous study may be related to the presence of more dietary residue in the small intestine. The highly digestible nature of the TEN formulas leaves little residue in the digestive tract, with outputs ranging from 7 to 85 g DM/d. Dry matter outputs from pigs fed a protein-free diet exceed 100 g/day (de Lange et al., 1989). While this in itself is not a large difference, it is important when considered in relation to body size. The highest daily dry matter output in this study was approximately 1 g/kg BW versus 2.5 g/kg BW in pigs,

thus more abrasion would be expected in the small intestine of pigs. Allen (1981) has proposed that proteolytic digestion and physical abrasion are the primary reasons for the presence of mucin in the lumen of the digestive tract. Support for this is provided by the observation that amino sugar output was influenced by the extremes of DM output (Figure III-5), but not by SF intake, which was the major determinant of DM output.

A role for the abrasive action of dietary fibers on mucin recovery is further supported by reports that fibers with insoluble components, such as wheat bran, cellulose and citrus fiber, illicit an increase in intestinal mucin secretion in rats while completely soluble fibers such as guar gum and carrageenan do not (Vahouny et al., 1985; Satchithanandam et al., 1989, 1990). In a separate study the concentration of immunoreactive mucin associated with the intestinal mucosa was observed to be lower in rats fed at a rate of one-half their normal daily intake, compared to control animals (Sherman et al., 1985). In light of observations made in this and previous studies (Vahouny et al., 1985; Satchithanandam et al., 1989, 1990) it appears that the lower mucin concentration could result from a smaller amount of residue in the digestive tract rather than malnourishment.

Mucin carbohydrate output was estimated from the composition of mucin carbohydrates after regression to zero SF intake and the content of GalNAc in ileal digesta. The daily output of mucin carbohydrate was approximatly 1.8 and 3.4 g in females and males, repectively, representing the majority of endogenous carbohydrates in ileal effluent (Table III-6). These results are consistant with observations in ileostomates fed NSP-free diets where galactose and fucose account for approximately 65% of endogenous neutral carbohydrates and their outputs suggest an output of 2 to 4 g of mucin carbohydrate (Englyst and Cummings 1985, 1986, 1987). Including protein, approximately 15%, maximum outputs of mucin in ileal digesta would be approximately 2 to 4 g/day. This is considerably lower than the 15 g reported by Clamp and Gough (1991). Diets were not presented in this former study, therefore, no comparison can be made. As dietary fiber consumption was increased the contribution of mucin

carbohydrates to total carbohydrates entering the large intestine declined to less than 10%, concurring with a previous suggestion that mucus contributes little to energy obtained from fermentation in the large intestine (McNeil, 1984).

This study sought to determine the effect of fiber supplementation of TEN formulas on the digestion of nutrients in the small intestine and on the output of mucin in ileal effluent. Increasing the consumption of soy fiber in enteral nutrition formulas did not impair the digestion of protein and carbohydrates and had little negative effect on the digestive tract itself as evidenced by a lack of effect on mucin output. The nutrients in Ensure and Enrich were also demonstrated to be highly digestible. Fiber supplemented enteral formulas should, therefore, be tolerated in the critically-ill patient. The higher output of endogenous components in males in this study was of interest and deserves further attention.

Table III-1. Characteristics of individuals participating in this study

Subject	Energy	Sex	Age	Height	Weight	BMI[a]	Colectomy
	(kcal/d)		(years)	(m)	(kg)	(kg/m^2)	(years)
1	1155	Female	51	1.57	49.9	20.2	8.0
2	1177	Female	23	1.73	91.2	30.5	3.0
3	1744	Female	48	1.59	44.0	17.4	0.7
4	1744	Female	47	1.74	65.2	21.5	10.0
5	1993	Male	53	1.65	82.0	30.1	3.0
6	1993	Male	68	1.80	88.2	27.2	5.0
7	2250	Male	37	1.56	55.3	22.7	0.5
8	2250	Male	43	1.73	82.8	27.7	18.0

[a]Body Mass Index.

Table III-2. Composition (g/100mL) of the enteral nutrition formulas

Item	ENSURE	ENRICH
Approximate analysis		
Dry Matter	20.17	22.18
Dietary Fiber[a]	0.10	1.65
Protein	3.72	4.00
Carbohydrate	10.47	11.79
Indispensible amino acids		
Arginine	0.13	0.14
Histidine	0.10	0.11
Isoleucine	0.19	0.20
Leucine	0.33	0.34
Lysine	0.25	0.26
Methionine	0.09	0.08
Phenylalanine	0.20	0.20
Threonine	0.15	0.16
Valine	0.23	0.24
Dispensible amino acids		
Alanine	0.12	0.13
Aspartic acid	0.27	0.30
Glutamic acid	0.80	0.82
Glycine	0.09	0.10
Serine	0.20	0.22
Tyrosine	0.15	0.13
Carbohydrates		
Arabinose	0.01	0.24
Fucose	0.02	0.04
Galactose	0.05	0.52
Glucose	9.90	10.37
Mannose	0.50	0.53
Rhamnose	trace	trace
Ribose	trace	trace
Xylose	trace	0.08

[a]According to Fredstrom et al. (1991).

Table III-3. The effect of the proportion of Enrich in the diet on the recovery (%) of dry matter and carbohydrate in ileostomy effluent

Item	TEST 1	TEST 2	TEST 3	TEST 4	TEST 5	SEM
Dry matter	8.1[a]	10.7[b]	11.8[b]	12.8[bc]	14.2[c]	0.61
Carbohydrate[e]	3.3[a]	6.3[b]	8.2[bc]	10.0[c]	13.3[d]	0.60
Arabinose	88.1[a]	122.6[b]	101.7[b]	102.9[b]	114.8[b]	29.87
Fucose	220.9	219.6	210.2	177.6	196.4	15.11
Galactose	208.7[a]	143.4[b]	109.4[b]	100.7[b]	110.7[b]	14.48
Glucose	1.6[a]	2.3[b]	3.0[bc]	3.4[c]	4.4[d]	0.22
Mannose	5.2[a]	6.5[ab]	7.5[ab]	8.4[b]	9.2[b]	0.71
Xylose	120.4	144.8	122.8	109.4	120.9	16.61

[abcd]Means in the same row with different superscripts differ ($P < 0.05$).
[e]Neutral carbohydrates.

Table III-4. Apparent and true ileal nitrogen and amino acid digestibilities (%)

	Apparent		True[a]
	Females	Males	
Nitrogen	86.8 ± 0.44[b]	83.9 ± 0.56[c]	92.9 ± 0.26
Indispensable amino acids			
Arginine	86.6 ± 0.44	84.3 ± 0.56	97.6 ± 0.26
Histidine	89.9 ± 0.33	88.3 ± 0.48	97.8 ± 0.18
Isoleucine	88.5 ± 0.33	86.9 ± 0.46[c]	92.1 ± 0.13
Leucine	91.4 ± 0.24	89.8 ± 0.38[c]	95.3 ± 0.13
Lysine	88.9 ± 0.40	87.5 ± 0.58	95.8 ± 0.18
Methionine	95.3 ± 0.16	94.1 ± 0.23[c]	97.7 ± 0.09
Phenylalanine	91.5 ± 0.24	89.5 ± 0.40[c]	95.1 ± 0.15
Threonine	79.9 ± 0.59	77.8 ± 0.81	90.5 ± 0.28
Valine	88.3 ± 0.32	86.4 ± 0.48[c]	93.3 ± 0.16
Total	88.9 ± 0.31	87.2 ± 0.47	94.7 ± 0.16
Dispensable amino acids			
Alanine	82.8 ± 0.48	79.2 ± 0.84[c]	89.2 ± 0.26
Aspartic acid	78.9 ± 0.65	75.8 ± 0.92	85.0 ± 0.27
Glutamic acid	90.6 ± 0.28	89.3 ± 0.38[c]	92.9 ± 0.12
Glycine	70.6 ± 0.99	66.9 ± 1.19	86.2 ± 0.46[d]
Serine	81.1 ± 0.62	80.2 ± 0.70	87.3 ± 0.24
Tyrosine	89.4 ± 0.31	87.4 ± 0.46[c]	95.2 ± 0.17
Total	85.6 ± 0.41	83.6 ± 0.57	90.4 ± 0.18

[a]Calculated by correcting for the daily outputs of N and amino acids in ileostomates consuming low N diets (Fuller et al., 1994).
[b]Mean ± SEM.
[c]Different from females ($P < 0.05$).
[d]Calculated from daily endogenous glycine output reported by Rowan et al. (1993).

Table III-5. Influence of the proportion of Enrich in the diet on the composition (mol/100mol) of protein and carbohydrates in crude mucin

	TEST 1	TEST 2	TEST 3	TEST 4	TEST 5	SEM
Amino acids						
Serine	12.0[a]	11.8[a]	11.8[a]	11.4[a]	10.8[b]	0.16
Aspartic acid	11.5[a]	11.9[b]	11.7[ab]	12.0[b]	12.1[b]	0.09
Glutamic acid	11.2[a]	11.0[a]	11.0[a]	11.0[a]	10.3[b]	0.15
Glycine	10.1[a]	10.2[ab]	10.1[a]	10.3[ab]	10.5[b]	0.11
Threonine	9.0[a]	9.2[a]	9.9[b]	9.8[b]	10.0[b]	0.1[illegible]
Rest	46.2	46.0	45.6	45.5	46.3	0.24
Carbohydrates						
Galactose	35.7[a]	37.3[ab]	38.2[ab]	39.4[ab]	41.5[b]	1.26
GluNAc[e]	24.3[a]	19.5[b]	17.0[b]	14.2[c]	13.8[c]	0.87
Fucose	16.7[a]	14.4[b]	13.9[bc]	12.5[bc]	12.0[c]	0.62
GalNAc[e]	9.2[a]	7.4[b]	6.6[bc]	5.6[c]	5.4[c]	0.39
Glucose	6.3[a]	3.6[b]	3.2[bc]	2.3[bc]	1.9[c]	0.39
Arabinose	3.0[a]	9.5[b]	12.1[c]	15.7[d]	15.6[d]	0.79
Mannose	2.4[a]	2.0[ab]	1.5[bc]	1.2[bc]	1.0[c]	0.23
Ribose	1.6[a]	3.0[b]	3.3[b]	4.1[c]	3.9[c]	0.19
Xylose	0.7[a]	3.4[b]	4.3[bc]	4.9[c]	4.8[c]	0.34

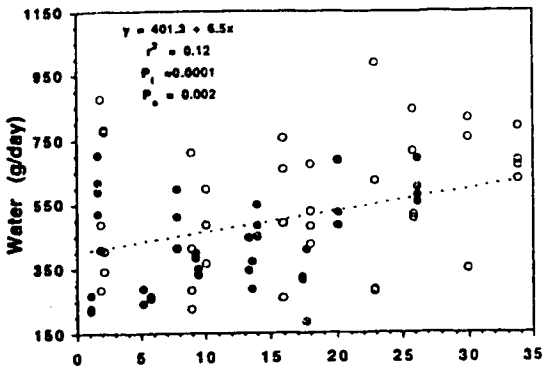
[abcd]Means in the same row with different superscripts differ ($P < 0.05$).
[e]N-acetylglucosamine and N-acetylgalactosamine.

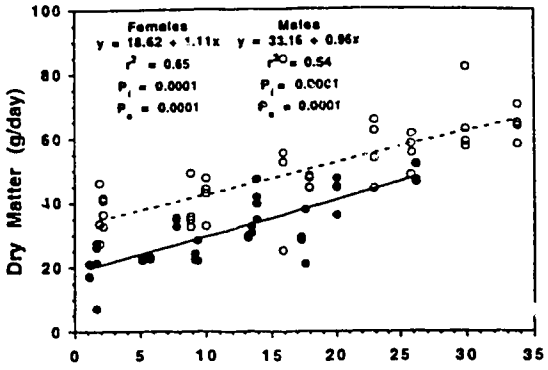
Table III-6. Outputs (g/day) of endogenous carbohydrates and mucin carbohydrates in ileal effluent and the contribution of mucin to endogenous carbohydrates[a]

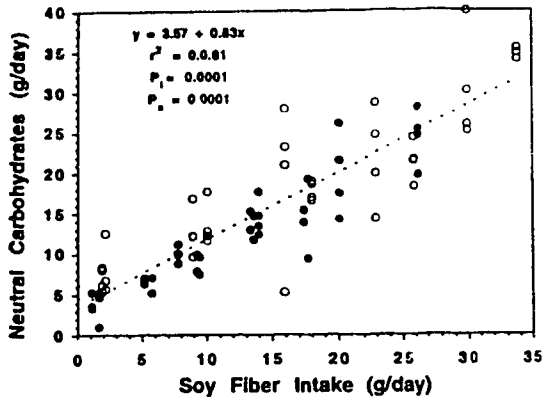
	Endogenous	Mucin	Contribution
Females			
Galactose	0.54 ± 0.261[b]	0.65 ± 0.074	120.37
Fucose	0.31 ± 0.049	0.27 ± 0.046	87.10
GalNAc[c]	0.21 ± 0.021	0.21 ± 0.023	100.00
GlcNAc[c]	0.77 ± 0.069	0.68 ± 0.072	87.01
Glucose	1.62 ± 0.290		
Mannose	0.28 ± 0.046		
Males			
Galactose	1.52 ± 0.491	1.35 ± 0.264	88.82
Fucose	0.67 ± 0.106	0.61 ± 0.062	91.04
GalNAc	0.35 ± 0.036	0.35 ± 0.043	100.00
GlcNAc	1.17 ± 0.106	1.04 ± 0.106	88.89
Glucose	2.61 ± 0.549		
Mannose	0.54 ± 0.078		

[a]Calculated by regression of carbohydrates from digesta (Figure III-2) and mucin (Figure III-4) to zero soy fiber intake.
[b]Mean ± SEM.
[c]N-Acetylgalactosamine and N-Acetylglucosamine.

Figure III-1. The effect of Soy fiber consumption in total enteral nutrition formulas on daily outputs of water, dry matter, neutral carbohydrate and nitrogen in ileal effluent of female (closed circles, solid lines) and male (open circles, dashed lines) subjects. Where only one line is presented intercept values are not different ($P > 0.05$) between sexes, $n = 40$ for both sexes except for carbohydrate output where $n = 39$ for females. Slopes were not different ($P > 0.40$) between sexes for any parameter.


y = 401.3 + 6.5x
r² = 0.12
P_l =0.0001
P_s = 0.002
Water (g/day)
1150
950
750
550
350
150
0
5
10
15
20
25
30
35


Females
y = 18.62 + 1.11x
r² = 0.65
P_l = 0.0001
P_s = 0.0001
Males
y = 33.16 + 0.86x
r² = 0.54
P_l = 0.0001
P_s = 0.0001
Dry Matter (g/day)
100
80
60
40
20
0
5
10
15
20
25
30
35


y = 3.57 + 0.83x
r² = 0.61
P_l = 0.0001
P_s = 0.0001
Neutral Carbohydrates (g/day)
40
35
30
25
20
15
10
5
0
0
5
10
15
20
25
30
35
Soy Fiber Intake (g/day)

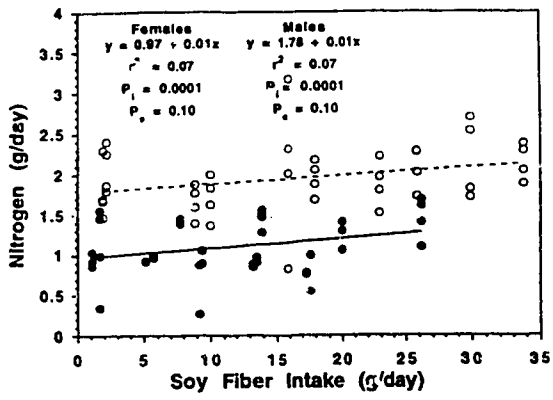

Females
y = 0.97 + 0.01x
r² = 0.07
P_l = 0.0001
P_s = 0.10
Males
y = 1.78 + 0.01x
r² = 0.07
P_l = 0.0001
P_s = 0.10
Nitrogen (g/day)
4
3.5
3
2.5
2
1.5
1
0.5
0
0
5
10
15
20
25
30
35
Soy Fiber Intake (g/day)

Figure III-2. The effect of soy fiber consumption on daily outputs of carbohydrates in ileal effluent of female (closed circles, solid lines) and male (open circles, dashed lines) subjects. Where only one line is presented intercept values are not different ($P > 0.05$) between sexes, n = 39 for females and 40 for males. Slopes were not different ($P > 0.10$) between sexes for any carbohydrate. N-Acetylgalactosamine outputs from one female subject (asterisks) were not included in calculations because they were higher th[illegible] (see text).

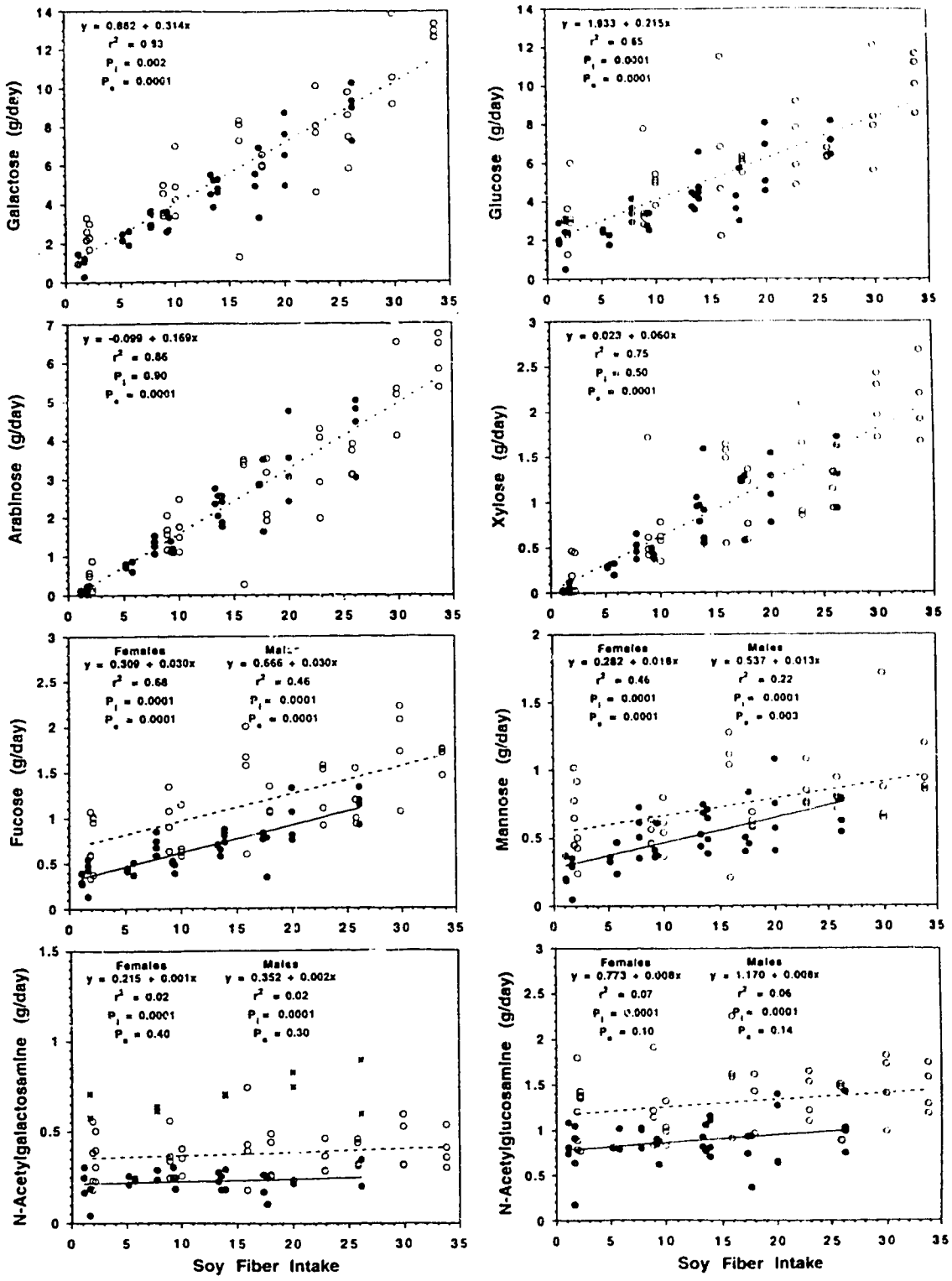
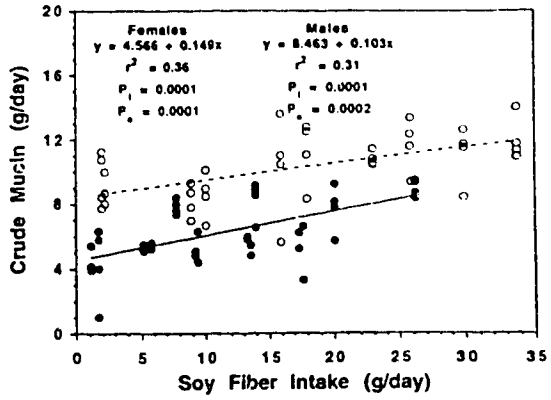
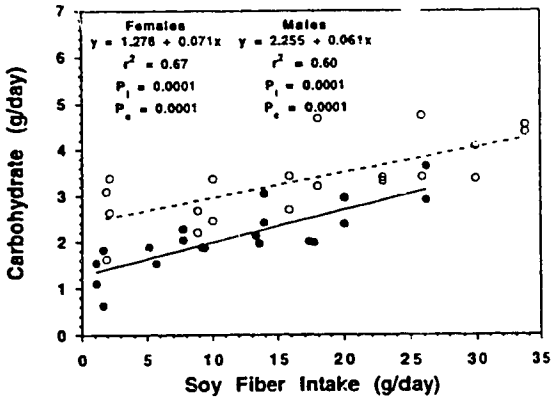

Galactose (g/day)
y = 0.882 + 0.314x
r² = 0.93
P_i = 0.002
P_e = 0.0001
Glucose (g/day)
y = 1.933 + 0.215x
r² = 0.65
P_i = 0.0301
P_e = 0.0001
Arabinose (g/day)
y = -0.099 + 0.169x
r² = 0.86
P_i = 0.90
P_e = 0.0001
Xylose (g/day)
y = 0.023 + 0.060x
r² = 0.75
P_i = 0.50
P_e = 0.0001
Fucose (g/day)
Females
y = 0.309 + 0.030x
r² = 0.68
P_i = 0.0001
P_e = 0.0001
Males
y = 0.666 + 0.030x
r² = 0.46
P_i = 0.0001
P_e = 0.0001
Mannose (g/day)
Females
y = 0.282 + 0.018x
r² = 0.46
P_i = 0.0001
P_e = 0.0001
Males
y = 0.537 + 0.013x
r² = 0.22
P_i = 0.0001
P_e = 0.003
N-Acetylgalactosamine (g/day)
Females
y = 0.215 + 0.001x
r² = 0.02
P_i = 0.0001
P_e = 0.40
Males
y = 0.352 + 0.002x
r² = 0.02
P_i = 0.0001
P_e = 0.30
N-Acetylglucosamine (g/day)
Females
y = 0.773 + 0.008x
r² = 0.07
P_i = 0.0001
P_e = 0.10
Males
y = 1.170 + 0.008x
r² = 0.06
P_i = 0.0001
P_e = 0.14
Soy Fiber Intake
Soy Fiber Intake

Figure III-3. The effect of soy fiber consumption on daily amounts of crude mucin in ileal effluent as well as carbohydrates and protein in crude mucin of female (closed circles, solid lines) and male (open circles, dashed lines) subjects. Slopes were not different ($P > 0.10$) between sexes, $n = 39$ for females and 40 for males for crude mucin and protein and $n = 20$ for both sexes for carbohydrate


Females
y = 4.566 + 0.149x
r² = 0.36
P_l = 0.0001
P_e = 0.0001
Males
y = 8.463 + 0.103x
r² = 0.31
P_l = 0.0001
P_e = 0.0002
Crude Mucin (g/day)
Soy Fiber Intake (g/day)


Females
y = 1.278 + 0.071x
r² = 0.67
P_l = 0.0001
P_e = 0.0001
Males
y = 2.255 + 0.061x
r² = 0.60
P_l = 0.0001
P_e = 0.0001
Carbohydrate (g/day)
Soy Fiber Intake (g/day)

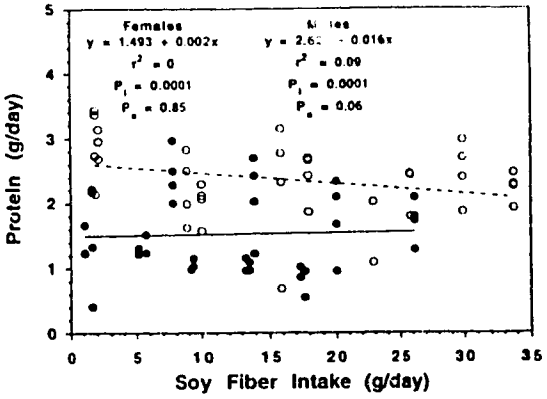

Females
y = 1.493 + 0.002x
r² = 0
P_l = 0.0001
P_e = 0.85
Protein (g/day)
Soy Fiber Intake (g/day)

Figure III-4. The effect of soy fiber consumption on the daily amounts of carbohydrates in crude mucin from female (closed circles, solid lines) and male (open circles, dashed lines) subjects. Where only one line is presented intercepts are not different ($P > 0.10$) between sexes. Slopes were not different ($P > 0.10$) between sexes for any carbohydrate, $n = 20$ for both sexes. N-Acetylgalactosamine outputs from one female subject (asterisks) were not included in calculations because they were higher than the rest (see text).

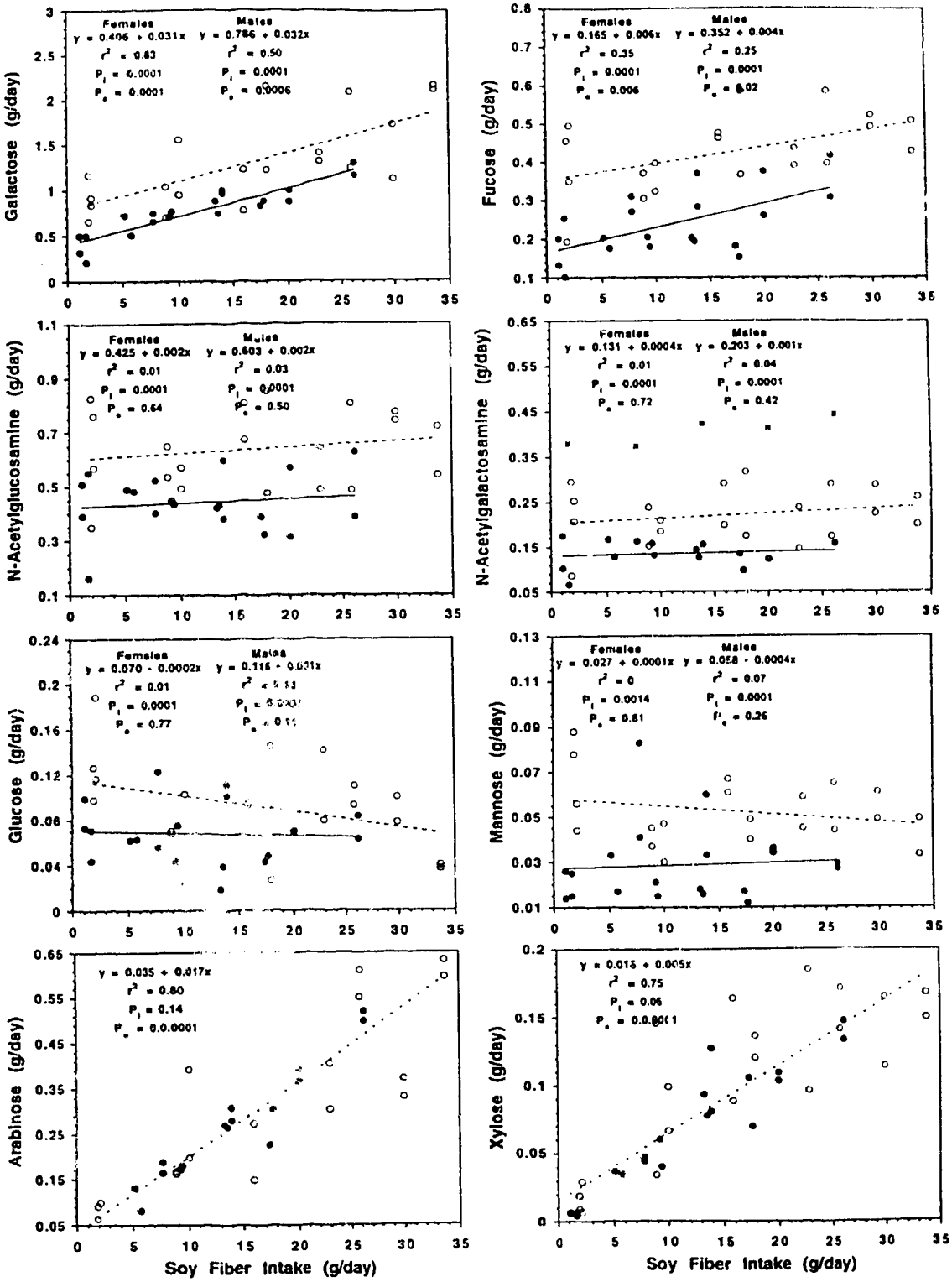
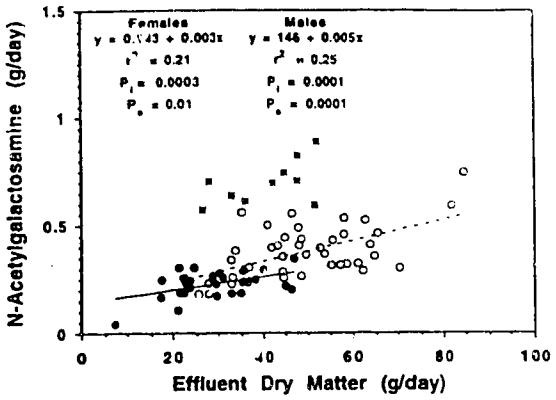
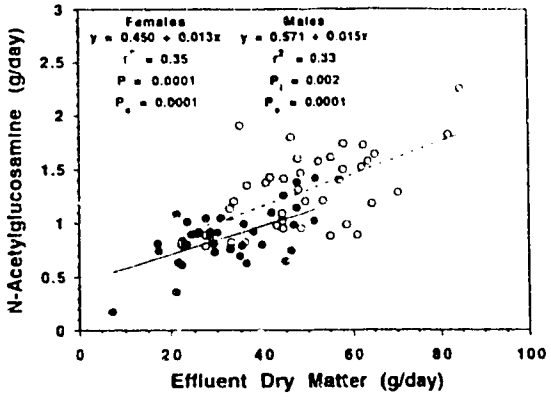

Galactose (g/day)
Females
y = 0.406 + 0.031x
r² = 0.83
P_l = 0.0001
P_e = 0.0001
Males
y = 0.786 + 0.032x
r² = 0.50
P_l = 0.0001
P_e = 0.0006
Fucose (g/day)
Females
y = 0.165 + 0.006x
r² = 0.35
P_l = 0.0001
P_e = 0.006
Males
y = 0.352 + 0.004x
r² = 0.25
P_l = 0.0001
P_e = 0.02
N-Acetylglucosamine (g/day)
Females
y = 0.425 + 0.002x
r² = 0.01
P_l = 0.0001
P_e = 0.64
Males
y = 0.603 + 0.002x
r² = 0.03
P_l = 0.0001
P_e = 0.50
N-Acetylgalactosamine (g/day)
Females
y = 0.131 + 0.0004x
r² = 0.01
P_l = 0.0001
P_e = 0.72
Males
y = 0.203 + 0.001x
r² = 0.04
P_l = 0.0001
P_e = 0.42
Glucose (g/day)
Females
y = 0.070 - 0.0002x
r² = 0.01
P_l = 0.0001
P_e = 0.77
Males
y = 0.116 - 0.001x
Mannose (g/day)
Females
y = 0.027 + 0.0001x
r² = 0
P_l = 0.0014
P_e = 0.81
Males
y = 0.058 - 0.0004x
r² = 0.07
P_l = 0.0001
P_e = 0.26
Arabinose (g/day)
y = 0.035 + 0.017x
r² = 0.80
P_l = 0.14
P_e = 0.0.0001
Xylose (g/day)
y = 0.015 + 0.005x
r² = 0.75
P_l = 0.06
P_e = 0.0.0001
Soy Fiber Intake (g/day)
Soy Fiber Intake (g/day)

Figure III-5. Effluent amino sugars in relation to dry matter output in female (closed circles, solid lines) and male (open circles, dashed lines) subjects. Slopes were not different ($P > 0.10$) between the sexes. N-Acetylgalactosamine outputs from one female subject (asterisks) were not included in calculations because they were higher than the rest (see text).


Females
y = 0.043 + 0.003x
r² = 0.21
P = 0.0003
P = 0.01
Males
y = 146 + 0.005x
r² = 0.25
P = 0.0001
P = 0.0001
N-Acetylgalactosamine (g/day)
Effluent Dry Matter (g/day)
0
0.5
1
1.5
0
20
40
60
80
100


Females
y = 0.450 + 0.013x
r² = 0.35
P = 0.0001
P = 0.0001
Males
y = 0.571 + 0.015x
r² = 0.33
P = 0.002
P = 0.0001
N-Acetylglucosamine (g/day)
Effluent Dry Matter (g/day)
0
0.5
1
1.5
2
2.5
3
0
20
40
60
80
100

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CHAPTER IV

THE EFFECT OF THE CONSUMPTION OF PEA FIBER ON ILEAL AND FECAL DIGESTIBILITIES AND ON MUCIN OUTPUT AT THE DISTAL ILEUM OF PIGS

A. Introduction

The recovery of endogenous protein is traditionally assumed to be related to dry matter intake and to be influenced by the consumption of fiber (Sauer et al., 1977; Taverner et al., 1981). However, Furuya and Kaji (1992) have recently provided argument that endogenous protein output is constant on a daily basis, relatively independent of dry matter or fiber intake. The use of protein-free diets is, however, subject to criticism (Sauer et al., 1977; Souffrant, 1991) particularily with respect to the effect of the physical properties of different protein-containing diets on endogenous secretions compared to that of protein-free diets.

The secretion of endogenous protein from a variety of sources has been the subject of recent reviews (eg., Low, 1982; Souffrant, 1991). Although different dietary components, particularily fiber, influence the secretion of endogenous protein, there is limited information concerning the recovery of these proteins at the distal ileum. Thus, in addition to providing necessary information for assessing the true value of a feedstuff, a determination of the recovery of endogenous protein, especially that from individual sources, at the distal ileum can provide important information regarding the effect of different feedstuffs in the digestive tract. In this respect the recovery of mucin is of interest for two reasons. Firstly, mucus lines the entire gastrointestinal tract and is, therefore, exposed to all of the chemical and physical forces of digestion. Allen (1981) has suggested that proteolytic degradation and physical abrasion are primary factors

responsible for erosion of mucus gels. Secondly, mucin undergoes little degradation once in the intestinal lumen (Hoskins, 1984, Chapter II). A measure of the recovery of mucin in ileal digesta might, therefore, provide insight into the effect of diet on the intestinal epithelium and on the recovery of endogenous protein at the distal ileum.

The consumption of 1 to 35 g soy fiber/d did not alter the output of mucin in ileal effluent from humans (Chapter III). However, the consumption of dietary fibers, including cellulose, wheat bran and citrus fiber have been reported to increase mucus synthesis in the gastrointestinal tract of rats (Vahouny, et al., 1985; Satchithanandam et al., 1989, 1990). The difference in these observations might be related to the relative amounts of dietary fiber consumed in relation to body weight, up to 0.5 g/kg BW versus approximately 10 g/kg BW in humans and rats, respectively, and thus on the amount of dietary residuals in the digestive tract. The purpose of this study was, therefore, to determine the effect of pea fiber (PF) consumption on ileal and fecal nitrogen, amino acid and carbohydrate digestibilities and on mucin output at the distal ileum of pigs.

B. Experimental Procedures

Animals and Diets.

A detailed description of the experimental procedures have been presented previously (McBurney and Sauer, 1992). In summary, four ileal-cannulated and four non-surgical control hybrid barrows (PIC), average initial BW 40 kg, were fed diets containing increasing levels of pea fiber in two 4x4 Latin square designs which were run simultaneously. Diets were formulated and fed to provide identical quantities of a wheat based diet (1600 g/d), in addition to 0, 80, 160 or 240 g PF/d (Table IV-1). Thus the animals were fed 1600, 1680, 1760 or 1840 g diet/d (as is) of the 0, 5, 10, and 15 g PF/100 g basal diet, respectively. Chromic oxide was included in the diet as a digestibility marker and vitamins and mineral

were supplied to NRC (1988) standards. Following a 7 d adaptation to the diet, feces were collected for 3 d from the ileal-cannulated pigs and for 5 d from the normal pigs. Ileal digesta were collected from the ileal-cannulated pigs for 2 consecutive d following the fecal collection period. Digesta and feces were frozen at -20 °C immediately following collection. The digesta were freeze-dried, pooled within pig and diet, ground through a .8 mm mesh screen in a model 4 laboratory Wiley mill and mixed prior to analyses.

Chemical Analyses

Analyses for DM and N were performed according to AOAC (1988) procedures. Chromic oxide was analyzed according to Fenton and Fenton (1979). Crude mucin (CM) was recovered from ileal digesta as described in Chapter II. Amino acids were determined in diets, ileal digesta, CM and feces by high performance liguid chromatography following acid hydrolysis in 6M HCl for 24 h (Jones and Gilligan, 1983). Preparation of samples for amino acid analysis was performed as described in the previous Chapter (III). Crude mucin (30 mg) was hydrolyzed in 6 mL 6M HCl to reduce the destruction of threonine and serine. Carbohydrates were analyzed in diets, digesta, CM and feces according to procedures described in Chapter II.

Calculations and Statistical Analyses

Digestibilies were calculated as described by Fan (1994). Daily mucin outputs in ileal digesta were calculated from the GlcNAc/GalNAc ratio in CM and the daily output of GalNAc in ieal digesta using the formulas presented in Chapter II.

Statistical analyses were performed using general linear models procedure (SAS, 1990). Experimental values were subjected to ANOVA for a 4 x 4 Latin square design and diet means were compared using the Student-Newman Keuls multiple range test (Steel and Torrie, 1980). Treatments means were analyzed using orthogonal polynomial regression procedures (Steel and Torrie, 1980).

There were no differences ($P > 0.10$) in fecal digestibilities between cannulated and uncannulated pigs, therefore, results were pooled within treatment. Multiple Linear regressions were performed using SAS regression procedures (SAS, 1990) using the following regression model presented by Goodlad and Mathers (1991):

$$Y = \alpha_1 X_1 + \alpha_2 X_2 + \alpha_3 X_1 X_3,$$

where Y was the output of carbohydrate in digesta or feces and X_1 and X_2 were intakes of carbohydrates from wheat and peas, respectively. X_3 has the value of 0 or 1 when peas were absent from or present in the diet, respectively, α_1 and α_2 are the recovery coefficients for carbohydrates for wheat and peas, respectively. The additional effect of the presence of peas on wheat carbohydrate recovery is given by α_3. Regression analyses were performed using SAS (1990) regression procedures as outlined by Steel and Torrie (1980).

C. Results and Discussion

The compositions of wheat, PF and the experimental diets are presented in Table IV-2. Nitrogen and amino acid contents of wheat were similar to that reported by NRC (1988). The carbohydrate content and composition was similar to that presented previously (Goodlad and Mathers, 1991; Gabert, 1994; Longland et al., 1993). Cellulose and hemicellulose represent approximately 1.5% and 7.5%, respectively, of the dry matter in wheat (Goodlad and Mathers, 1991; Gabert, 1994). Arabinoxylans, a hemicellulose, comprises the majority of wheat fiber (Selvendran, 1984; Bach Knudsen and Hansen, 1991). The composition of PF closely resembled that reported by Titgemeyer et al. (1991) and that of pea hulls presented by Selvendran (1984). Pea fiber contained 4.7% crude protein and 81.3% carbohydrates on a dry matter basis. Uronic acid, which accounts for about 15% of PF dry matter (Selvendran, 1984; Titgemeyer et al., 1991), was not analyzed in this study. Pea fiber is predominantly an insoluble fiber, comprised mostly of cellulose (Selvendran, 1984; Titgemeyer et al., 1991).

The apparent ileal and fecal DM and carbohydrate digestibilities are presented in Table IV-3. Overall carbohydrate digestibility in the basal diet, 87.1%, was a balance between a high glucose and relatively low nonstarch polysaccharide (NSP) digestibilities. The digestibility of wheat starch is expected to exceed 97% since the digestibility of glucose (93.5%) includes NSP glucose which accounts for approximately 2.5% of wheat dry matter (Goodlad and Mathers, 1991; Longland et al., 1993) and is undigested in the small intestine (Bach Knudsen and Hansen, 1991). Ileal starch digestibilities in cereal based diets are typically in excess of 95% (Graham et al., 1986; Bach Knudsen and Hansen, 1991; Bach Knudsen et al., 1993).

The ileal digestibilities of arabinose and xylose, the predominant NSP carbohydrates in wheat, were 24.2% and 31.2%, respectively. These values are higher than that previously reported for arabinoxylans, -10 to 16%, in cereal-based diets fed to pigs (Graham et al., 1986; Bach Knudsen and Hansen, 1991; Bach Knudsen et al., 1993; Gabert, 1994). The high digestibility of arabinoxylans for wheat might represent an adaptation of the small intestinal microflora to wheat NSP since ileal arabinose digestibilities increased ($P < 0.05$) from period 1 to period 4. Arabinose and xylose digestibilities in the first period were 7.5 and 17.9%, respectively, compared to 30.0 and 29.8%, respectively, in the fourth period. Fecal arabinose, xylose and galactose digestibilities decreased ($P < 0.05$) from period 1 to period 4. Since these carbohydrates are derived predominantly from wheat, an increase in their outputs in feces indicates an adaptation of the large intestinal microflora to the more readily fermentable PF (Table IV-4), thus reducing fermentation of wheat NSP. Longland et al. (1993) reported an increase in the fecal digestibility of NSP from Solka-floc fed to pigs at 6 weeks compared to 2 weeks.

The apparent fecal digestibilities of carbohydrates for the basal diet were up to twice that observed at the distal ileum (Table IV-3), indicating that considerable fermentation had occured in the large intestine. The fecal digestibility of glucose for the basal diet was 97.5%. Since only traces of starch

and ß-glucans from wheat are recovered in feces (Bach Knudsen et al., 1991), the fecal glucose is likely derived from cellulose. The fecal digestibilities of arabinose and xylose in wheat, 58.9% and 72.5%, respectively, were similar to that observed previously (Bach Knudsen et al., 1991; Goodlad and Mathers, 1991). The ileal and fecal digestibilities of fucose, galactose, mannose and ribose are confounded by their low contents in the basal diet and their presence in endogenous secretions and bacteria (Graham et al., 1986; Chapter II).

Increasing the consumption of pea fiber from 0 to 240 g/d resulted in a linear decrease ($P < 0.05$) in ileal DM, galactose, glucose, mannose and ribose digestibilities. Although not significant, ileal arabinose and xylose digestibilities also declined. Glucose digestibility also exhibited a quadratic response ($P = 0.04$) to increasing pea fiber consumption. The effect of increasing PF consumption on fecal digestibilities was more variable. There was a linear decrease ($P < 0.05$) in DM and total carbohydrate as well as glucose and mannose digestibilities, while arabinose, fucose, ribose and xylose digestibilities increased linearly ($P < 0.05$). Fecal galactose digestibilities were unaffected by the consumption of PF. The contrasting effects of PF consumption on carbohydrate digestibilities in the small and large intestines are the result of the relative digestibilities of wheat and PF in these areas of the digestive tract.

The ileal and fecal carbohydrate digestibilities for wheat and PF, determined by MLR, are presented in Table IV-4. As expected, ileal and fecal digestibilities for wheat were similar to those in the basal diet. Ileal digestibilities for PF were negative and, with the exception of mannose, not different ($P > 0.10$) from zero. The low digestibilities of fucose, galactose and mannose reflect increases in the output of endogenous carbohydrates as a result of increasing the consumption of PF. The digestibility of glucose for PF is of interest since the output of glucose in ileal digesta increases quadratically (thus the significant quadratic decrease in ileal digestibility) rather than linearly as the MLR model assumes. The slope of glucose intake versus output is, therefore, skewed upwards and the digestibility of glucose for PF is overestimated by MLR. The

digestibilities of glucose in the diets, derived by correcting the intake and output of glucose for glucose consumed with pea fiber, were 93.5, 93.7, 93.7 and 90.2% (SEM = 0.64) in pigs consuming 0, 80, 160 and 240 g PF/d. Corrected glucose digestibilities were different ($P < 0.05$) only with the diet containing 240 g PF/d, indicating an impaired digestion and/or absorption of wheat starch in this diet since glucose is not a primary constituent of endogenous carbohydrate (Englyst and Cummings, 1985, 1986). Insoluble fiber can reduce the absorption of nutrients by decreasing the intestinal transit rate and limiting the time available for absorption (Kritchevsky, 1988). Pharmaceutically increasing or slowing gastrointestinal transit has been shown to increase and decrease, respectively, the recovery of starch in ileal effluent (Holgate and Read, 1983; Chapman et al., 1985).

Fecal digestibilities of arabinose, xylose and glucose for PF were 104.9±13.97, 97.9±10.58 and 94.4±1.69%, respectively, indicating a complete fermentation of PF in the large intestine. Stanogias and Pearce (1985) and Goodlad and Mathers (1991) reported fecal digestibilities of noncellulosic polysaccharides in pigs to be 100% for diets supplying 75 or 150 g NDF/kg diet DM from pea hulls and for pea , respectively. The complete digestion of glucose from PF is, however, somewhat surprising since fecal cellulose digestibilities for peas and pea hulls in pigs range from 52 to 73% (Stanogias and Pearce, 1985; Goodlad and Mathers, 1991), similar to that observed after 48 h in fecal incubation (Titgemeyer et al., 1991). The high fecal cellulose digestibility for PF in this study may be related to processing since the PF used in this study was a fine powder while the majority of the pea hull preparation used by Stanogias and Pearce (1985) consisted of particles which were > 0.5 mm. Van Soest (1984) reported that fine grinding of wheat bran increased ($P < 0.05$) intestinal transit from 39 to 52 h to 64 to 68 h in humans. While not significant, Van Dokkum et al. (1983) observed a similar increase, from 44 h with course-bran to 62 h for fine-bran. An increase in retention time might be expected to illicit a higher overall fermentation, however, Nyman and Asp (1985) observed no effect of particle size on the recovery of NSP carbohydrates from wheat bran in the feces of rats.

Results presented in Table IV-4 indicate that, while no digestion of PF occurred in the small intestine, it was completely fermented in the large intestine. On the other hand, wheat fiber NSP was digested to similar extents in the small and large intestines. The effect of increasing the consumption of PF on carbohydrate outputs at the distal ileum and in feces, and thus on carbohydrate digestibilities, is illustrated in Figure IV-1. All carbohydrates increased linearly ($P < 0.02$) with increasing PF consumption. The rates of increase (g/g PF consumed per d) for most carbohydrates were similar to their contents in PF, further indicating that PF was quantitatively recovered in ileal digesta. Fecal arabinose and xylose, the predominant NSP carbohydrates in wheat, were unaffected ($P > 0.20$) by the consumption of PF. Daily fecal glucose output increased only moderately, 5.5 g/100 PF per day, demonstrating that PF was largely fermented in the large intestine. Therefore, increasing the consumption of PF will result in the decrease in ileal carbohydrate digestibilities and the increase in fecal carbohydrate digestibilities observed in Table IV-3. An increase in fecal carbohydrate digestibilities was observed in pigs fed increasing amounts of peas with wheat (Goodlad and Mathers, 1991). The recovery of PF NSP at the distal ileum also accounts for the decline in DM digestibilities (Chapter III). Linear increases ($P < 0.0001$) in fecal mannose, fucose, galactose, ribose and rhamnose with increasing PF intake is of interest since, considering the complete digestion of PF, they are likely to derived from either endogenous sources or bacteria.

The apparent ileal digestibilities of N and amino acids in the experimental diets is presented in Table IV-5. Crude protein digestibility in the basal diet was 82.0%. Amino acid digestibilities ranged from 68.7% (glycine) to 91.9% (methionine). These values were within the range presented previously for wheat (Sauer and Ozimek, 1986; Fan, 1994). The low digestibilities of threonine and glycine, relative to the other amino acids, is attributed to their prevalence in endogenous secretions (Chapter II; Sauer and Ozimek, 1986). Increasing the consumption of PF from 0 to 240 g/d resulted in a linear decrease ($P < 0.05$) in

apparent ileal N and amino acid digestibilities. These results are consistant with the findings of numerous studies which have been summarized in recent reviews (Fernandez and Jorgensen, 1986; Sauer and Ozimek, 1986; Low, 1985). Amino acid digestibilities were, however, significantly lower only at the highest level of PF consumption.

While it is clear from this, and previous studies, that the consumption of fiber results in an increase in the output of N and amino acids at the distal ileum, their source is yet to be determined. In studies with humans, in which the typical consumption of fiber is less than 50 g/d, it appears that the output of N consumed with fiber is largely responsible for the increase in ileal N outputs (eg., Ellegard and Bosaeus, 1991). The complete recovery of PF NSP at the distal ileum, indicating that little disruption of the fiber matrix had occured, suggests that the recovery of N and amino acids from PF might also contribute to the declining ileal N and amino acid digestibilities. To address this, the daily outputs of N and amino acids are presented in Table IV-6. Daily outputs increased linearly ($P < 0.05$) with increasing consumption of PF, however, similar to digestibilities, differences between diets were significant only at the highest level of PF inclusion. With the exception of disproportionate increases in the contributions of glutamate and glycine, as the consumption of PF was increased from 0 to 240 g/d, the relative proportions of amino acids recovered at the distal ileum did not appear to be affected by the different diets. Although the basis for the low outputs of N and amino acids with the consumption of 160 g PF/d is unclear, the proportions of these components are consistant with this pattern. This pattern was also reflected in the maximum contributions of PF N and amino acids to changes in the daily outputs of N and amino acids, respectively, and in changes in the minimum outputs of non-PF N and amino acids (Table IV-6) as a result of increasing the consumption of PF.

The results presented in Table IV-6 indicate that higher recoveries of wheat, PF and endogenous N and amino acids are responsible for the decline in apparent ileal digestibilities resulting from increasing the consumption of PF. The presence

of wheat protein in ileal digesta from pigs consuming the basal diet, and its increase with the consumption of increasing amounts of PF, is inferred from the high contribution of glutamate to the total output of amino acids at the distal ileum. The contribution of glutamate to total amino acids in PF (Table IV-2) and endogenous protein (e.g., Furuya and Kaji, 1992; Chapter II), is considerably lower than that observed in wheat (Table IV-2). That increasing the consumption of PF increases the output of endogenous protein at the distal ileum is expressed by the disproportionate increase in daily glycine output, relative to other amino acids, with increasing consumption of PF. Glycine represents the major source of N in bile secretions (Sambrook, 1981) and it is clear that dietary fibers are capable of sequestering bile acids, increasing their delivery to the large intestine (Kritchevsky, 1988). Thus, the increased output of glycine in ileal digesta is consistant with the increased recovery of PF NSP. In addition, increasing the consumption of PF also leads to an increase in the daily output of mucin (Table IV-11). Although the digestibilities of PF N and amino acids is unknown, it is reasonable to assume that at least a portion of them would be recovered in ileal digesta and that the overall recovery would increase with increasing consumption of PF.

Fecal crude protein and amino acid digestibilities (Table IV-7) for wheat were, on average, 5.4 percentage units higher in feces than at the distal ileum. The largest increases were observed for glycine and threonine, 17.5 and 8.3 percentage units, respectively. The smallest increases were observed for isoleucine and methionine, 1.7 and 1.2 percentage units, respectively, while there was a net synthesis of lysine (-3.1 percentage units). These results, which are consistant with previous data, are attributed to microbial degradation of amino acids and the synthesis of microbial protein (Sauer and Ozimek, 1986). Apparent fecal digestibilities decreased linearly ($P < 0.001$) with increasing consumption of PF and exhibited more discrete differences between diets. The consumption of 60 and 80 g PF/d lowered ($P < 0.05$) fecal digestibilities compared to the basal diet and the consumption of 240 g PF/d resulted in fecal digestibilities which

were lower ($P < 0.05$) than all other consumption levels. A decline in fecal digestibilities with increased fiber consumption has been observed in several previous studies (e.g., Stanogias and Pearce, 1985; Sauer et al., 1991).

The effect of fiber consumption on apparent fecal nitrogen and amino acid digestibilities is related to the supply of fermentable carbohydrates to the large intestine. This relationship is demonstrated in Table IV-8. The disappearance of all carbohydrates in the large intestine, from 77.9 to 278.3 g/d overall, increased ($P < 0.01$) with increasing PF consumption. The disappearance of N and amino acids was generally unaffected ($P > 0.05$) by the consumption of PF, although there was a numerical decline in the disappearance of several amino acids. At the highest level of PF consumption there was a net synthesis of isoleucine, lysine, methionine, aspartic acid and tyrosine, which are prominant amino acids in bacterial protein (Dugan, 1992). As with carbohydrates, the disappearance of arginine, glutamic acid and glycine increased with increasing PF consumption but this was significant for glycine only.

The relative disappearances of amino acids in the large intestine are consistant with observations that bacterial protein represents the majority of the protein in feces (Stephen and Cummings, 1980; Sauer et al., 1991; Mosenthin et al., 1992). Increasing the supply of fermentable substrate has two important effects on bacterial metabolism; firstly there is a decrease in the breakdown of amino acids and secondly there is an increase in the assimilation of ammonia into bacterial protein (Wrong, 1988). Recently Mortensen et al. (1990) demonstrated that the addition of lactulose to human fecal incubation systems completely inhibited the degradation of amino acids, albumin and blood. In addition, several studies (e.g., Stephen and Cummings, 1979; Sauer et al., 1991) have reported an increase in fecal bacterial nitrogen excretion following the consumption of fiber. These results are consistant with fermentation of these substrates as indicted by an increase in fecal bacterial nitrogen excretion following the infusion of starch into the large intestine (Gargallo and Zimmerman, 1981; Mosenthin et al., 1992). The lower fecal digestibilities, resulting from increasing consumption of PF are

due to both a reduction in the microbial degradation of endogenous and dietary protein in the large intestine and an increase in the fecal excretion of bacterial protein. Goodlad and Mathers (1991) reported linear increases in fecal DAPA following graded intakes of peas.

The amino acid and carbohydrate compositions of CM were not different ($P > 0.05$) between diets, therefore, only the average composition is presented in Table IV-9. The importance of mucin protein was demonstrated by high contents of threonine (13.5±0.38 mol/100mol) and serine (11.3±0.09 mol/100mol), which along with proline are the predominant amino acids in mucus (Allen, 1981; Neutra and Forstner, 1987; Mantle and Allen, 1989). However, similar to previous studies (Chapters II and III), CM preparations contained a large proportion of nonmucus protein. The recovery of nonmucin protein (sum of amino acids) in CM, 2.7, 4.5, 4.2 and 5.1 g/d following the consumption of diets containing 0, 80, 160 and 240 g PF/d, respectively, is of interest since it exhibits a pattern similar to that of the daily output of amino acids in ileal digesta. The composition of nonmucin protein in CM was not different ($P > 0.10$) between diets, therefore, only the mean composition is presented in Table IV-10. Aside from a higher proportion of nonmucin protein, versus mucin protein, the composition of the nonmucin protein in CM was similar to that observed in Chapter II. Two inferences are made from these observation; firstly, the high contributions of threonine and serine indicate that, as in Chapter II, the contribution of mucin protein was underestimated and, secondly, that the nonmucin protein was predominantly endogenous. Arabinose and xylose were the predominant carbohydrates (79.4±0.51 mol/100mol) indicating the presence of soluble NSP derived from the diet (Chapter III), primarily from wheat.

Daily total, gastric and intestinal mucin outputs in ileal digesta were estimated from the GluNAc/GalNAc ratio in CM and the daily output of GalNAc in ileal digesta (Chapter II). The daily ileal output of mucin from pigs given the basal diet, 6.2 g/d (Table IV-11), was similar to that from pigs fed a protein-free diet while continuously administered either saline or a complete amino acid

mixture intravenously, 5.6 and 6.0 g/d, respectively (Chapter II). Any conclusions regarding the validation of feeding protein-free diets to estimate endogenous protein from these results must be made with caution since, despite the similar outputs of mucin, feeding a wheat versus a protein-free diet apparently has very different consequences in the digestive tract. Gastric mucin represented approximately 25% of the mucin from pigs fed a protein-free diet compared to about 45% for the basal wheat diet in this study.

Daily outputs of mucin at the distal ileum were not different ($P > 0.05$) between the diets, however, there was a trend ($P < 0.15$) towards an increase in total, gastric and intestinal mucin with increasing PF consumption. Much of the variation in these estimates resulted from one pig (Figure IV-2). Excluding this pig, daily mucin output increased linearly ($P = 0.005$) with increasing PF consumption; 6.1, 6.9, 7.3 and 7.8 g/d for diets containing 0, 80, 160 and 240 g PF/d, respectively. The daily ileal outputs of gastric and small intestinal mucin also increased linearly in these three pigs as the consumption of PF was increased, although this was significant for small intestinal mucin only ($P = 0.03$). An increase in the output of mucin with the consumption of PF is in contrast to results with protein-free diets which suggest that the daily output of endogenous protein is relatively independent of fiber intake (Furuya and Kaji, 1992), although mucin does represents less 10% of ileal endogenous protein in pigs fed protein-free diets (Chapter II). In the former study (Furuya and Kaji, 1992), however, threonine output did exhibit the highest rate of increase with increasing cellulose consumption at 1.5 mg/g NDF/d.

Gastic mucus secretion is stimulated by many of the same neural and hormonal processes controlling the digestive process (Neutra and Forstner, 1987; Mantle and Allen, 1989). Kowalewski et al. (1976) reported a 44% increase in mucin carbohydrate output from canine Heidenhain pouches following the consumption of food. However, Mantle and Allen (1989) have suggested that some of the stimulatory effect of these secretagogues may be secondary to their effect on pepsin secretion which increases degradation of the mucus gel (Allen,

1981; Neutra and Forstner, 1987; Mantle and Allen, 1989). In addition Allen (1981) suggests that physical abrasion augments the effect of proteolysis on mucus gels. The increase in luminal mucin following the consumption of 5% citrus fiber, but not 5% guar gum (Satchithanandam et al., 1990) suggests that proteolysis and physical abrasion may indeed be important determinants of the output of gastric mucin. Both fibers are predominantly soluble and thus have potential negative effects on enzyme activity (Kritchevsky, 1988). However, citrus fiber is approximately 25% insoluble and the abrasive action of this component, brought about by the churning of gastric contents, may explain the higher rate of gastric mucin secretion with this fiber. Similarly, increased abrasion as a result of increasing the consumption of PF may explain the increase in gastric mucin output in this study. Although the consumption of fiber can influence gastrointestinal hormones (Kritchevsky, 1988) and presumably neural controls, via its effect on distention of the stomach, it is unclear wether graded increases in fiber consumption potentiate these actions.

An increase in the abrasive capacity of digesta, as a result the recovery of PF is also proposed to account for an increase in small intestinal mucin output (Chapter III). Higher rates of mucus synthesis and secretion in the small intestine following adaptation to diets containing the insoluble fibers cellulose and wheat bran (Vahouny et al., 1985; Satchithanandam et al., 1989) and the partially insoluble citrus fiber (Satchithanandam et al., 1990) compared to fiber-free diets have been reported. However, consumption of soluble fibers, guar gum (Satchithanandam et al., 1990) and carrageenan (unpublished data referenced by Satchithanandam et al., 1990), do not influence the secretion of small intestinal mucin. In the previous Chapter (III) a relationship between between mucin and dry matter outputs was observed. An increase in mucin output at the distal ileum with increasing PF consumption indicates an increase in the erosion of gastrointestinal mucus gels. However, This erosion is apparently balanced by a higher rate of mucus secretion (Vahouny, et al., 1985; Satchithanandam et al., 1989, 1990) and the integrity of the mucus gel, and thus its capacity to protect the

underlying mucosal epithelium, is likely to be maintained. This is supported by the fact that no visible damage was observed in jejunal villi of rats consuming 15% cellulose or bran compared to those fed chow (Casssidy et al., 1981).

Hoskins (1984) has summarized much of the evidence showing that degradation of mucin occurs largely by microbes in the large intestine. This is supported by the considerable disappearance of amino sugars in the large intestine in this study, 1.37 and 0.73 g/d for GlcNAc and GalNAc, respectively (Table IV-8). A similar disappearance of amino sugars was observed in the large intestine of preruminant calves fed milk, bean and soya diets (Combe et al., 1980). Fermentation of mucin in the large intestine would explain the considerable relative disappearance of threonine in the large intestine in this and previous studies (Sauer and Ozimek, 1986).

The daily output of amino sugars in feces increased with increasing consumption of PF (Figure IV-2), however, the daily disappearances of amino sugars in the large intestine was not affected (Table IV-8). It is possibe that all mucin arriving from the small intestine was completely fermented and that the increase in fecal amino sugars represent compounds originating in the large intestine, possibly from colonic mucin or bacteria. However, studies in rats (Shiau and Chang, 1983; Shiau and Yo, 1992) indicate that at least some of the fecal amino sugars may represent the recovery of gastric and/or small intestinal mucin in feces. Decreases in the specific activity and total output of mucinase in the feces of rats fed a variety of dietary fibers was consistant with an increase in the fermentability of the different fibers (Shiau and Chang, 1983). In the more fermentable fibers, pectin and guar gum, the output of mucinase was lower, although not significantly, with consumption of 15% versus 5% fiber. In a further study (Shiau and Yo, 1992) a decrease in total and specific fecal mucinase activity was observed in conjunction with an increase in the proportion of cellulose in the diet. The complete fermentation of mucin requires several species of bacteria contributing different ezymes (Hoskins, 1984) and since many of these are capable of using different substrates (Bayliss and Houston, 1984) it is likely that they will

choose the more readily fermentable source. However, the relative rates of increase of fecal carbohydrates (Figure IV-1) suggest that the increase in fecal carbohydrates might be predominantly due to an increase in bacterial mass, rather than mucin output. Fecal ribose and rhamnose outputs, 0.38 and 0.07 g/100 g PF intake per d, respectively, are indicative of an increase in bacterial mass since they are important constituents of bacterial carbohydrates (Walla et al., 1984; Gilbert et al., 1988). Since PF was completely fermented and the digestion of wheat NSP in the digestive tract was unaffected by PF consumption, extra outputs of ribose and rhamnose must be derived from bacteria or possibly some other endogenous source.

The purpose of this study was to determine the effect increasing the consumption of PF on apparent ileal and fecal protein and carbohydrate digestibilities and the daily output of mucin at the distal ileum of pigs. Taking into account the recovery of PF constituents in ileal digesta, it is apparent that the digestibility of nutrients from wheat and the recovery of endogenous constituents were influenced by the consumption of PF. Increasing the consumption of PF results in an increase in the output of both gastric and intestinal mucin in ileal digest.

Table IV-1. Composition (g/100g basal diet) of the experimental diets

Ingredient	Wheat +0 g PF	Wheat +80 g PF	Wheat +160 g PF	Wheat +240 g PF
Wheat	96.50	96.50	96.50	96.50
Pea fiber	0.00	5.00	10.00	15.00
Iodized salt	0.50	0.50	0.50	0.50
Calcium carbonate	1.35	1.35	1.35	1.35
Dicalcium phosphate	1.10	1.10	1.10	1.10
Vitamin mixture[1]	0.20	0.20	0.20	0.20
Mineral mixture[1]	0.10	0.10	0.10	0.10
Chromic oxide	0.25	0.25	0.25	0.25
Total	100.00	105.00	110.00	115.00

[1]The vitamin and mineral mixtures provided the following per kg basal diet: 5.2 mg retinyl palmitate; 375 µ cholecalciferol; 44 mg all-*rac*-α-tocopherol acetate; 2 mg menadione; 2.2 mg riboflaven; 12 mg niacin; 11 mg pantothenicacid; 11 µg vitamin B_{12}; 550 mg choline; 1.1 mg thiamin; 1.1 mg pyridoxine; 100 µg biotin; 6 mg folic acid; 50 mg Fe; 50 mg Zn; 2 mg Mn; 3 mg Cu; 140 µg I; 150 µg Se.

Table IV-2. Composition (% DM) of pea fiber, wheat and the experimental diets

Item	Pea fiber	Wheat	Diets (level of pea fiber, g/d) 0	80	160	240
Dry Matter	92.53	85.41	85.82	86.23	86.73	87.05
Nitrogen	0.76	2.86	2.72	2.61	2.54	2.42
Carbohydrates	81.26	81.20	77.69	80.08	78.77	76.55
Indispensable Amino Acids						
Arginine	0.29	0.72	0.70	0.70	0.66	0.66
Histidine	0.10	0.37	0.37	0.36	0.34	0.32
Isoleucine	0.20	0.60	0.60	0.58	0.55	0.54
Leucine	0.29	1.01	1.00	0.97	0.94	0.90
Lysine	0.30	0.46	0.46	0.46	0.43	0.44
Methionine	0.00	0.19	0.19	0.17	0.16	0.14
Phenylalanine	0.21	0.77	0.74	0.73	0.71	0.68
Threonine	0.19	0.49	0.47	0.46	0.45	0.44
Valine	0.22	0.69	0.64	0.64	0.61	0.59
Dispensable Amino Acids						
Alanine	0.23	0.62	0.59	0.60	0.55	0.55
Aspartic Acid	0.42	0.89	0.85	0.86	0.82	0.82
Glutamic Acid	0.51	5.63	5.34	5.19	4.85	4.61
Glycine	0.21	0.73	0.71	0.68	0.65	0.64
Serine	0.20	0.73	0.69	0.68	0.64	0.63
Tyrosine	0.13	0.38	0.39	0.36	0.37	0.35
Carbohydrates						
Arabinose	6.44	2.55	2.48	2.81	2.90	2.89
Fucose[a]	0.23	0.03	0.02	0.03	0.04	0.05
Galactose	2.29	0.64	0.63	0.70	0.74	0.76
Glucose	57.73	73.58	70.28	71.43	69.67	66.99
Mannose	0.65	0.28	0.28	0.30	0.29	0.31
Rhamnose	trace	trace	trace	trace	trace	trace
Ribose	1.58	0.15	0.15	0.22	0.28	0.34
Xylose	12.35	3.99	3.87	4.62	4.87	5.27

[a]Fucose content in wheat and diets estimated from fucose content in PF and diet 4.

Table IV-3. Apparent ileal and fecal dry matter and carbohydrate digestibilities (%) in pigs consuming increasing amounts of pea fiber

Item	Pea fiber intake (g/day)				
	0	80	160	240	SEM[a]
Ileal					
Dry Matter[b]	76.0[d]	69.7[e]	66.8[e]	56.4[f]	1.30
Carbohydrate[b]	87.1[d]	82.5[e]	78.8[f]	71.4[g]	0.90
Carbohydrates					
Arabinose	24.2	23.1	23.0	13.3	4.13
Fucose	-90.9	-78.9	-55.5	-61.9	12.92
Galactose[c]	35.4[d]	23.5[de]	34.1[d]	21.5[e]	2.75
Glucose[b]	93.5[d]	89.9[e]	86.2[f]	79.4[g]	0.60
Mannose[c]	41.3	25.8	31.3	22.3	4.28
Ribose[b]	50.7[d]	31.1[e]	27.2[e]	15.9[e]	3.59
Xylose	31.2	27.4	25.0	22.3	4.84
Fecal					
Dry Matter[b]	87.2[e]	85.7[f]	85.7[f]	84.3[g]	0.23
Carbohydrate[c]	94.6	94.2	94.1	93.5	0.27
Carbohydrates					
Arabinose[d]	58.9[e]	60.4[ef]	63.4[ef]	66.0[f]	1.54
Fucose[c]	30.7[e]	31.9[e]	40.5[f]	37.3[ef]	2.15
Galactose	74.2	72.8	74.2	73.0	0.53
Glucose[d]	97.5[e]	97.3[e]	97.1[ef]	96.5[f]	0.21
Mannose[c]	84.2	82.6	81.9	81.9	0.69
Ribose[c]	62.6[e]	67.4[ef]	72.7[f]	68.9[ef]	1.74
Xylose[b]	72.5[e]	75.1[ef]	77.4[fg]	79.8[g]	1.16

[a]Standard error of the mean (n = 16).
[b]Linear effect ($P < 0.001$). Glucose also quadratic ($P = 0.04$).
[c]Linear effect ($P < 0.05$).
[defg]Means in the same row with different superscripts differ ($P < 0.05$).

Table IV-4. Apparent ileal and fecal carbohydrate digestibilities (%) in wheat and pea fiber, determined by multiple linear regression

Item	Wheat	Pea Fiber	α_3[a]
Ileal			
Arabinose	29.5 ± 6.76[b]	-0.5 ± 40.46	0.02 ± 0.123
Fucose	-79.2 ± 46.46	-23.5 ± 79.20	0.12 ± 0.849
Galactose	40.1 ± 6.05	-15.1 ± 43.25	0.06 ± 0.110
Glucose	93.8 ± 0.66	-24.3 ± 9.97	-0.02 ± 0.012
Mannose	43.2 ± 3.94	-30.9 ± 30.94	0.03 ± 0.072
Ribose	48.0 ± 13.24	-11.0 ± 15.05	-0.11 ± 0.242
Xylose	37.8 ± 5.02	9.4 ± 22.32	0.05 ± 0.092
Fecal			
Arabinose	61.8 ± 2.33	104.7 ± 13.97	0.07 ± 0.043
Fucose	34.9 ± 5.99	45.8 ± 10.21	0.01 ± 0.109
Galactose	76.4 ± 0.91	72.4 ± 6.48	0.02 ± 0.017
Glucose	97.6 ± 0.11	94.4 ± 1.69	0.00 ± 0.002
Mannose	84.7 ± 0.96	80.7 ± 7.57	0.02 ± 0.018
Ribose	57.9 ± 4.81	68.9 ± 5.47	-0.07 ± 0.088
Xylose	75.1 ± 2.38	97.9 ± 10.58	0.03 ± 0.043

[b]The effect of the consumption of pea fiber on the output of nutrients from wheat, g/g pea fiber consumed.
[a]Standard error of the mean.

Table IV-5. Apparent ileal nitrogen and amino acid digestibilities (%) in pigs consuming increasing amounts of pea fiber

Item	Pea fiber intake (g/day)				
	0	80	160	240	SEM[a]
Nitrogen[b]	82.0[e]	78.0[f]	77.8[f]	70.4[g]	0.95
Indispensable Amino Acids					
Arginine[c]	84.4[e]	81.7[e]	82.3[e]	76.9[f]	0.96
Histidine[c]	86.5[e]	82.5[ef]	83.2[ef]	79.1[f]	1.10
Isoleucine[c]	84.0[e]	80.6[ef]	80.8[ef]	77.2[f]	1.01
Leucine[c]	84.5[e]	81.2[ef]	81.7[ef]	77.4[f]	1.02
Lysine	77.8	72.2	73.9	70.6	1.88
Methionine[b]	91.9[e]	89.2[f]	88.3[f]	85.5[g]	0.76
Phenylalanine[d]	85.6	82.9	82.5	78.7	1.69
Threonine[c]	74.8[e]	69.6[ef]	69.5[ef]	66.3[f]	1.31
Valine[c]	79.8[e]	75.9[ef]	76.3[ef]	70.0[f]	1.59
Dispensable Amino Acids					
Alanine[c]	73.6[e]	69.4[ef]	69.6[ef]	64.0[f]	1.65
Aspartic Acid[c]	76.2[e]	71.6[ef]	72.1[ef]	68.0[f]	1.44
Glutamic Acid[b]	93.6[e]	92.3[e]	92.3[e]	89.2[f]	0.47
Glycine[b]	68.7[e]	59.9[e]	58.1[e]	46.4[f]	2.46
Serine[c]	83.2[e]	80.0[ef]	80.2[ef]	75.9[f]	1.14
Tyrosine[c]	82.9[e]	78.5[e]	79.9[e]	74.6[f]	1.09

[a]Standard error of the mean (n = 16).
[b]Linear effect ($P < 0.001$).
[c]Linear effect ($P < 0.01$).
[d]Linear effect ($P < 0.05$).
[efgh]Means in the same row with different superscripts differ ($P < 0.05$).

Table IV-6. Total and nonpea fiber[a] nitrogen and amino acid outputs (g\d) at the distal ileum of pigs consuming increasing amounts of pea fiber (PF) and the maximum contribution (%) of pea fiber to ileal outputs

	Daily output					PF Contribution			Non-PF		
PF Intake (g/d)	0	80	160	240	SEM[b]	80	160	240	80	160	240
Nitrogen[f]	6.94[c]	8.45[d]	8.66[d]	11.66[e]	0.371	36.87	64.70	35.55	0.96	0.61	3.04
Indispensable Amino Acids											
Arginine[f]	1.50[c]	1.84[c]	1.79[c]	2.43[d]	0.100	61.07	144.48	68.16	0.14	-0.13	0.30
Histidine[g]	0.68[c]	0.90[cd]	0.86[cd]	1.06[d]	0.056	33.69	82.31	59.12	0.15	0.03	0.16
Isoleucine[g]	1.31[c]	1.63[cd]	1.62[cd]	1.96[d]	0.085	46.93	97.31	69.65	0.17	0.01	0.20
Leucine[g]	2.12[c]	2.64[cd]	2.62[cd]	3.24[d]	0.145	41.48	87.32	58.14	0.31	0.06	0.47
Lysine[h]	1.39[c]	1.87[cd]	1.71[cd]	2.08[d]	0.128	46.78	138.52	97.37	0.25	-0.12	0.02
Methionine[g]	0.21[c]	0.27[cd]	0.28[cd]	0.33[d]	0.018	0.00	0.00	0.00	0.05	0.07	0.11
Phenylalanine[h]	1.46	1.80	1.89	2.32	0.181	45.48	71.30	53.40	0.18	0.12	0.40
Threonine[g]	1.63[c]	2.05[d]	2.09[d]	2.37[d]	0.090	34.39	62.11	58.56	0.27	0.18	0.31
Valine[g]	1.77[c]	2.22[cd]	2.22[cd]	2.85[d]	0.148	36.10	72.58	45.12	0.29	0.12	0.59
Dispensable Amino Acids											
Alanine[g]	2.13[c]	2.64[c]	2.55[c]	3.17[d]	0.140	32.59	78.91	48.33	0.35	0.09	0.54
Aspartic Acid[g]	2.79[c]	3.52[cd]	3.48[cd]	4.22[d]	0.180	42.55	90.44	65.30	0.42	0.07	0.50
Glutamic Acid[f]	4.69[c]	5.82[c]	5.72[c]	7.95[d]	0.350	32.95	72.31	34.39	0.76	0.29	2.14
Glycine[f]	3.05[c]	3.97[d]	4.18[d]	5.52[e]	0.247	16.79	27.32	18.74	0.77	0.82	2.01
Serine[g]	1.59[c]	1.97[c]	1.95[c]	2.45[d]	0.112	38.51	82.11	52.02	0.24	0.07	0.41
Tyrosine[g]	0.92[c]	1.14[c]	1.13[c]	1.43[d]	0.061	42.46	85.95	54.16	0.13	0.03	0.24

[a]Output minus the intake from pea fiber.
[b]Stadard error of the least squared means.

Table IV-7. Apparent fecal nitrogen and amino acid digestibilities (%) in pigs consuming increasing amounts of pea fiber

Item	Pea fiber intake (g/day)				
	0	80	160	240	SEM[a]
Nitrogen[b]	87.8[c]	84.8[d]	82.4[e]	77.9[f]	0.77
Indispensable Amino Acids					
Arginine[b]	91.0[c]	88.7[d]	87.7[d]	85.1[e]	0.73
Histidine[b]	92.8[c]	90.8[d]	89.9[d]	87.1[e]	0.64
Isoleucine[b]	85.7[c]	80.9[d]	78.5[d]	72.9[e]	1.14
Leucine[b]	88.6[c]	85.2[d]	83.6[d]	79.5[e]	0.80
Lysine[b]	74.7[c]	66.9[cd]	61.2[d]	52.7[e]	2.61
Methionine[b]	93.1[c]	89.6[d]	87.2[d]	81.5[e]	0.95
Phenylalanine[b]	90.4[c]	87.5[d]	86.2[d]	83.0[e]	0.60
Threonine[b]	83.1[c]	77.4[d]	74.7[d]	68.2[e]	1.37
Valine[b]	86.3[c]	82.7[d]	80.5[d]	76.1[e]	0.91
Dispensable Amino Acids					
Alanine[b]	81.2[c]	76.2[d]	72.8[d]	66.1[e]	1.38
Aspartic Acid[b]	81.7[c]	76.3[d]	73.2[d]	67.5[e]	1.46
Glutamic Acid[b]	96.4[c]	95.3[d]	94.7[d]	93.3[e]	0.30
Glycine[b]	86.2[c]	81.6[d]	79.7[d]	74.8[e]	1.04
Serine[b]	90.8[c]	88.1[d]	86.5[d]	83.6[e]	0.64
Tyrosine[b]	86.1[c]	81.3[d]	79.6[d]	75.0[e]	1.24

[a]Standard error of the mean (n = 16).
[b]Linear effect (P = 0.0001).
[cdef]Means in the same row with different superscripts differ (P < 0.05).

Table IV-8. Disappearance (g/d) of dry matter, nitrogen, amino acids and carbohydrates in the large intestine of pigs consuming increasing amounts of pea fiber

Item	Pea fiber intake (g/day)				
	0	80	160	240	SEM[a]
Dry matter[b]	154.79[e]	234.72[f]	290.01[f]	453.67[g]	17.510
Nitrogen	2.65	3.11	2.15	2.80	0.629
Carbohydrate[b]	77.89[e]	137.43[f]	185.47[g]	278.32[h]	10.385
Indispensable Amino Acids					
Arginine	0.73	0.79	0.69	0.86	0.143
Histidine	0.35	0.46	0.39	0.40	0.078
Isoleucine	0.27	0.14	-0.02	-0.36	0.208
Leucine	0.71	0.69	0.49	0.30	0.259
Lysine[d]	-0.01	-0.18	-0.50	-1.31	0.394
Methionine	0.06	0.03	0.01	-0.08	0.046
Phenylalanine	0.56	0.56	0.51	0.48	0.210
Threonine	0.66	0.64	0.54	0.12	0.210
Valine	0.69	0.74	0.56	0.59	0.222
Dispensable Amino Acids					
Alanine	0.78	0.72	0.50	0.22	0.291
Aspartic Acid	0.88	0.79	0.48	-0.08	0.435
Glutamic Acid	2.35	2.56	2.17	3.03	0.467
Glycine[d]	1.84	2.27	2.32	2.93	0.286
Serine	0.81	0.87	0.75	0.77	0.185
Tyrosine	0.25	0.21	0.08	-0.02	0.168
Carbohydrates					
Arabinose[c]	11.30[e]	15.69[f]	17.69[f]	24.74[f]	1.699
Fucose[c]	0.48[e]	0.65[ef]	0.74[fg]	0.92[g]	0.055
Galactose[b]	3.45[e]	5.07[f]	4.69[f]	6.73[g]	0.291
Glucose[b]	37.49[e]	77.46[f]	116.57[g]	188.03[h]	6.319
Mannose[b]	1.64[e]	2.23[f]	2.35[f]	3.07[g]	0.103
Rhamnose	0.17	0.25	0.23	0.21	0.046
Ribose[b]	0.14[e]	1.02[f]	1.88[g]	2.86[h]	0.201
Xylose[b]	21.09[e]	32.77[f]	39.36[fg]	49.74[g]	3.085

[a]Standard error of the mean (n = 16).
[bcd]Linear effect: [b]($P < 0.001$), [c]($P < 0.01$),[d]($P < 0.05$).
[efgh]Means in the same row with different superscripts differ ($P < 0.05$).

Table IV-9. Composition of crude mucin from ileal digesta of pigs fed increasing amounts of pea fiber

Item	Composition
Protein (mol/100mol)	
Threonine	13.46 ± 0.380
Glycine	12.11 ± 0.140
Serine	11.34 ± 0.091
Glutamic Acid	10.67 ± 0.089
Aspartic Acid	10.12 ± 0.137
Alanine	9.52 ± 0.069
Valine	7.20 ± 0.033
Leucine	6.92 ± 0.047
Isoleucine	4.03 ± 0.043
Lysine	3.39 ± 0.062
Arginine	3.26 ± 0.052
Phenylalanine	3.03 ± 0.043
Tyrosine	2.67 ± 0.040
Histidine	1.53 ± 0.036
Methionine	0.75 ± 0.020
Carbohydrate (mol/100mol)	
Ribose	0.86 ± 0.088
Rhamnose	0.85 ± 0.092
Fucose	1.73 ± 0.197
Arabinose	30.85 ± 0.199
Xylose	48.55 ± 0.524
Mannose	0.63 ± 0.043
Glucose	3.21 ± 0.253
Galactose	8.32 ± 0.299
N-Acetylglucosamine	2.74 ± 0.092
N-Acetylgalactosamine	2.27 ± 0.128

Table IV-10. Composition (g/100g amino acids) of nonmucin protein in crude mucin from ileal digesta of pigs fed increasing amounts of pea fiber

Item	Composition
Indispensable Amino Acids	
Arginine	5.07 ± 0.070
Histidine	2.04 ± 0.050
Isoleucine	4.52 ± 0.045
Leucine	8.16 ± 0.057
Lysine	4.39 ± 0.067
Phenylalanine	4.43 ± 0.060
Threonine	9.56 ± 0.280
Valine	6.98 ± 0.032
Dispensable Amino Acids	
Alanine	7.65 ± 0.072
Aspartic Acid	12.36 ± 0.125
Glutamic Acid	14.04 ± 0.133
Glycine	8.05 ± 0.110
Serine	8.49 ± 0.092
Tyrosine	4.29 ± 0.057

Table IV-11. Daily output (g) of total, gastric and intestinal mucin in ileal digesta of pigs consuming increasing amounts of pea fiber assuming mucin is either completely proteolytically degraded of completely undegraded (native)[a]

Item	Pea fiber intake (g/day)				
	0	80	160	240	SEM[a]
Degraded					
Total	5.87	6.88	6.41	7.37	0.444
Gastric	2.70	3.32	3.20	3.28	0.216
Intestinal	3.17	3.56	3.21	4.09	0.301
Native					
Total	6.18	7.25	6.76	7.78	0.469
Gastric	2.82	3.46	3.34	3.42	0.224
Intestinal	3.38	3.80	3.42	4.58	0.322

[a]Determined from the GlcNAc/GalNAc ratio in crude mucin and the daily output of GalNAc in ileal digesta (Chapter II).

Figure IV-1. The effect of increasing the consumption of pea fiber on daily ileal (closed circles, solid line) and fecal outputs (open circles, dashed line) of neutral carbohydrates. All intercepts ($P < 0.001$) and, with the exception of ileal rhamnose and fecal arabinose and xylose, all slopes ($P < 0.01$) were significant.

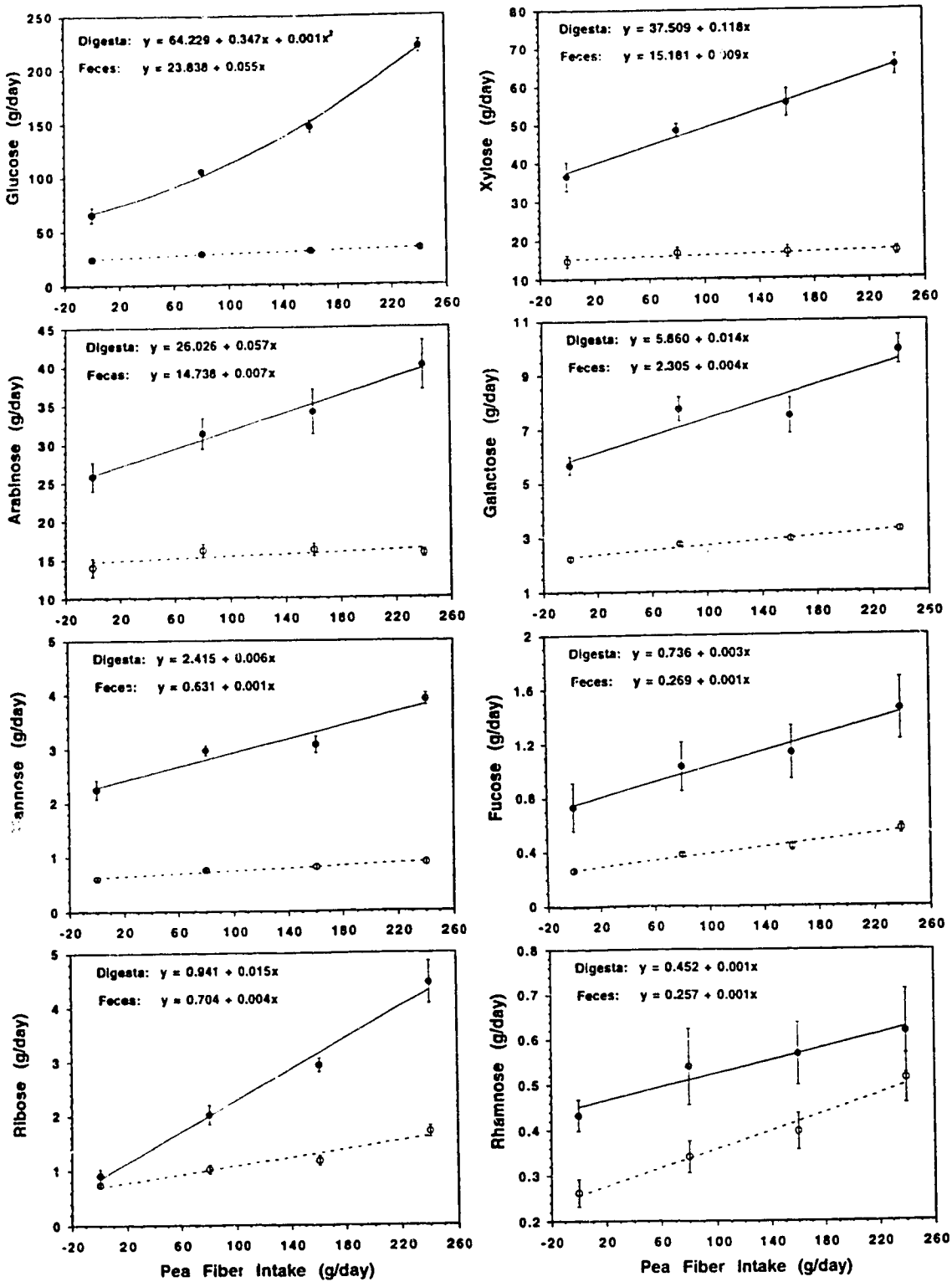

Glucose (g/day)
Digesta: y = 64.229 + 0.347x + 0.001x²
Feces: y = 23.838 + 0.055x
Xylose (g/day)
Digesta: y = 37.509 + 0.118x
Feces: y = 15.181 + 0.009x
Arabinose (g/day)
Digesta: y = 26.026 + 0.057x
Feces: y = 14.738 + 0.007x
Galactose (g/day)
Digesta: y = 5.860 + 0.014x
Feces: y = 2.305 + 0.004x
annose (g/day)
Digesta: y = 2.415 + 0.006x
Feces: y = 0.631 + 0.001x
Fucose (g/day)
Digesta: y = 0.736 + 0.003x
Feces: y = 0.269 + 0.001x
Ribose (g/day)
Digesta: y = 0.941 + 0.015x
Feces: y = 0.704 + 0.004x
Rhamnose (g/day)
Digesta: y = 0.452 + 0.001x
Feces: y = 0.257 + 0.001x
Pea Fiber Intake (g/day)
Pea Fiber Intake (g/day)

Figure IV-2. The effect of increasing the consumption of PF on the daily output of total, gastric and intestinal mucin in ileal digesta of pigs.

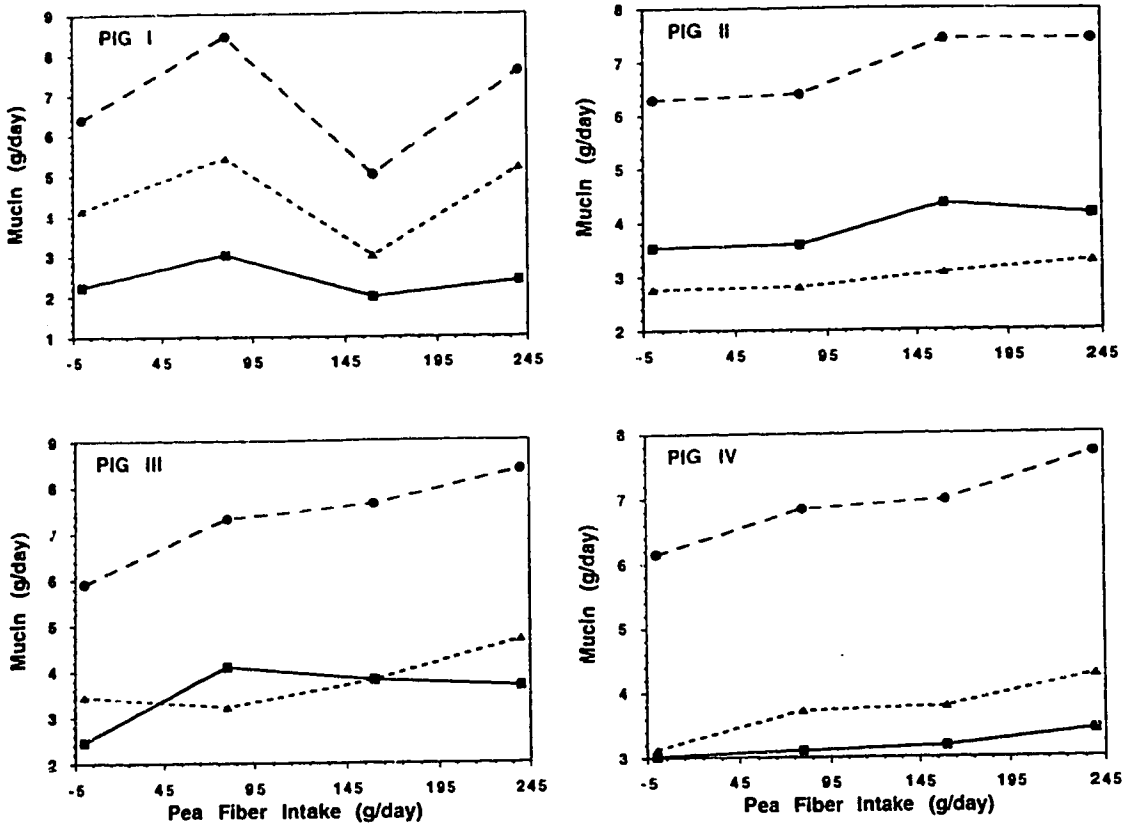
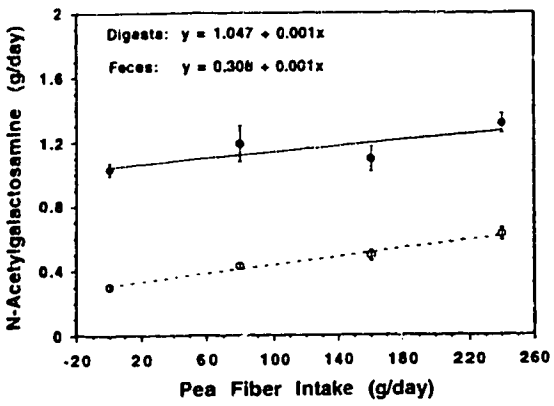
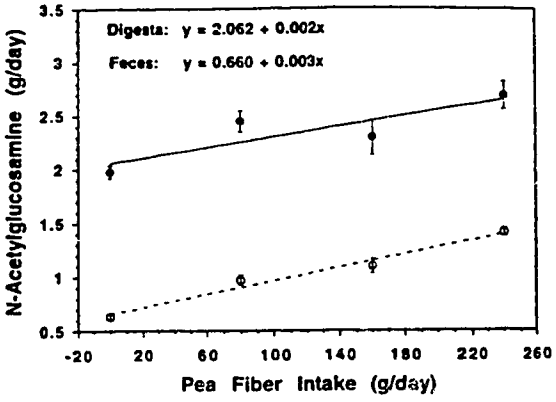

PIG I
PIG II
PIG III
PIG IV
Mucin (g/day)
Pea Fiber Intake (g/day)
-5
45
95
145
195
245

Figure IV-3. The effect of increasing the consumption of pea fiber on ileal and fecal amino sugar outputs. Except for ileal GalNAc (P_{slope} = 0.06), all intercepts and slopes are significant (P < 0.001).


Digesta: y = 1.047 + 0.001x
Feces: y = 0.308 + 0.001x
N-Acetylgalactosamine (g/day)
Pea Fiber Intake (g/day)
0
0.4
0.8
1.2
1.6
2
-20
20
60
100
140
180
220
260


Digesta: y = 2.062 + 0.002x
Feces: y = 0.660 + 0.003x
N-Acetylglucosamine (g/day)
Pea Fiber Intake (g/day)
0.5
1
1.5
2
2.5
3
3.5
-20
20
60
100
140
180
220
260

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CHAPTER V

EVALUATION OF THE ^{15}N-ISOTOPE DILUTION TECHNIQUE FOR DETERMINING THE RECOVERY OF ENDOGENOUS PROTEIN IN ILEAL DIGESTA OF PIGS: THE EFFECT OF DILUTION IN THE ACTUAL PRECURSOR POOL FOR ENDOGENOUS N SECRETION

A. Introduction

The ^{15}N-isotope dilution technique was proposed to be a valid method for determining the recovery of endogenous protein at the distal ileum of pigs fed protein-containing diets (Souffrant et al., 1986; de Lange et al., 1992). The recovery is measured by dilution of the ^{15}N-enrichment in ileal digesta, relative to that in the precursor pool for endogenous protein synthesis. Since plasma free amino acids are presumed to be precursors for endogenous protein synthesis, the enrichment in the trichloroacetic acid (TCA)-soluble pool is expected to be similar to that in endogenous protein secreted into the digestive tract.

Studies using the ^{15}N-isotope dilution technique (de Lange et al., 1990), suggest that most of the protein recovered at the distal ileum is of endogenous origin. These recoveries were most likely overestimated since the real digestibilities of several amino acids exceeded 100% (de Lange et al., 1990). These authors, therefore, proposed that dilution in protein *per se* would better estimate the recovery of endogenous protein.

The objective of the present study was to evaluate the ^{15}N-isotope dilution technique. The first part of this study attempts to discern the effect of using the dilution of ^{15}N in total N, as opposed to that in protein, for determining the recovery of endogenous protein at the distal ileum.

B. Experimental Procedures

Animals and Diets

Six barrows, average initial BW 33 kg, were surgically fitted with a re-entrant cannula at the distal ileum according to procedures adapted from Sauer (1976). Following surgery, the barrows were housed individually in stainless steel metabolic crates in a temperature-controlled barn (20 to 22°C). During recovery from surgery the pigs were fed increasing amounts of the experimental diet (Table 1), until they consumed 700g twice daily at 0800 and 2000, which was continued throughout the experiment. Water was freely available from a low-pressure drinking nipple. At least 12 d following cannulation, catheters made from Silastic medical grade tubing (Dow Corning Corp., Midland, MI) were surgically implanted into each of the external jugular veins of three of the barrows according to procedures described by Edwards (1980). The 1st three barrows consuming their allotted meals, 700g twice daily, within 1 h were selected selected for catheterization. Sedation was brought about by an intramuscular injection (2 mL) of Stresnil (Janssen Pharmaceutical, Beerse, Belgium) and naesthesia was maintained with an intravenous injection (5 mL) of Hypnorm (Janssen Pharmaceutical). The barrows were allowed to recover for at least 4 days prior to initiation of the saline infusion. The remaining three barrows were later used as donors for the reinfusion of digesta into the large intestine of the experimental pigs.

The diet was formulated to contain barley as the sole source of protein (Table 1). Sucrose was included to improve palatability. Canola oil was included to reduce dustiness. Vitamins and minerals were supplemented according to NRC (1988) standards. Chromic oxide was included to determine nutrient digestibilities.

Following recovery from surgery, infusion was initiated with sterile saline 1 d prior to an 8 d continuous intravenous infusion of [^{15}N]leucine (99% ^{15}N-

enrichment; MSD Isotopes, Pointe Claire, Que) via the right jugular catheter. Approximately 30 mg ^{15}N-leucine, dissolved in sterile saline, was infused per kg BW per day. Solutions were prepared as described by de Lange et al. (1990), and infused at a rate of approximately 500 mL per day via a peristaltic pump (Ismatic MP 13, Ismatic S.A., Limmastrasse 107/109, Zurich, Switzerland). Blood samples were taken hourly, starting at the beginning of the saline infusion and continued for 8 d following the initiation of the [^{15}N]leucine infusion. Approximately 2 mL of blood was withdrawn from the left catheter and combined with 2 mL of heparinized saline. Samples were immediately centrifuged at 1,000 x g for 10 min. The supernatant was removed and immediately frozen. Hourly digesta collections were performed on d 3 and 8 of the [^{15}N]leucine infusion period. Twelve (0800 to 2000 and 2000 to 0800) and 24 h (0800 to 0800) pooled digesta samples were prepared by combining 30 and 20%, respectively, of each hourly sample. Digesta from the donor pigs were infused at a rate of 50% of the hourly output. Digesta samples were frozen at -20 °C immediately after each hourly collection.

Euthanesia was performed by the administration of 2 mL of T-61 euthanesia solution (Hoechst Canada Inc., Montreal, Que.) into one of the catheters. This experiment was approved by the Animal Policy and Welfare Committee, Faculty of Agriculture and Forestry, University of Alberta. Animals were cared for in accordance with guidelines outlined by CCAC (1980).

Chemical Analyses

Prior to analyses, plasma samples were thawed and pooled over 12 h by combining 1 mL from each sample between 0800 and 2000, and between 2000 and 0800. Fifteen percent TCA (2 mL) was added to 4 mL aliquots of pooled plasma and the TCA-soluble and insoluble fractions separated by centrifugation at 3,000 x g for 10 min. The TCA-insoluble fraction was washed twice with 2 mL 5% TCA. All TCA-soluble fractions were combined and brought to 10 mL with 5% TCA. The TCA-insoluble fractions were freeze-dried prior to further analysis.

Amino acid analyses in the TCA-soluble and insoluble fractions of plasma and the 12 and 24 h pooled digesta samples were performed by high-pressure liquid chromatography (Varian 5000) according to the method of Jones and Gilligan (1983). Samples were prepared for amino acid analysis as described by Li et al. (1994). Proline was estimated by gas chromatography-mass spectrometry (GC-MS), using leucine in the sample as the internal standard. Nitrogen analysis was performed by the Kjeldahl method according to AOAC (1980) procedures. Chromic oxide was analyzed by the method of Fenton and Fenton (1979). Plasma urea was determined using a Blood Urea Nitrogen kit (Sigma Chemicals, St. Louis, MO)

Samples were prepared for determination of ^{15}N-enrichment in total N as described by de Lange et al. (1990, 1992). The distillate remaining after N analysis was transferred to a Kjeldahl flask and 20 mL 40% sodium hydroxide and zinc chips added. The ammonia was then redistilled into a beaker containing 50 mL .01M HCl. The water was evaporated in an oven at 60°C and the ammonium chloride salts recovered by solubilization in 15 mL distilled water. Samples were frozen and transported to Rostock, Germany where they were analyzed using an Emission Spectrometer (Isonitromat RFT5201, VEB Statron, Fuerstenwalde, F.R.G.).

Samples were prepared for ^{15}N analysis in amino acids according to a modification of the procedure described by de Lange et al. (1992). Trichloroacetic acid was removed from plasma samples by three consecutive extractions into equal volumes of ether and the sample acidified with the addition of concentrated HCl. Following acid hydrolysis in 6M HCl for 24 h, the TCA-insoluble fraction of plasma and ileal digesta were diluted to approximately .1M HCl with the addition of water. Aliquots of the dilute acid hydrolysates and TCA soluble fraction of plasma, containing approximately 20 µg of leucine, were applied to 2 mL AG 50-X8 cation exchange resin (200 to 400 mesh, hydrogen form; Bio-Rad Laboratories, Richmond, C.A. 94804) placed in 3 mL syringes from which the plunger was removed. Samples were washed on, and the column rinsed, with 10 mL deionized water to remove neutral and negatively charged organic

compounds. Amino acids were eluted with 6mL of freshly prepared 4M NH_4OH and freeze-dried. Trimethylsilyl derivatives were prepared with the addition of 50 µL N,O-bis-trimethyl-trifluoracetamide (Sigma Chemical) and 50 µL of acetonitrile (Sigma Chemical) as described by Gehrke and Leimer (1971) and the samples heated at 110°C for 30 min.

The ^{15}N-enrichments in amino acids were determined by single ion monitoring (SIM) GC-MS (Hewlett Packard 5890 Series II gas chromatograph connected to a Hewlett Packard 5971A mass selective detector). Approximately .5 µL of sample was injected onto a DB5 gas chromatograph column (.32 mm i.d. x 30 m; J&W Scientific, Folsom, C.A. 95630). Helium was used as the carrier gas at a flow rate of 1 mL/sec. Column temperature, initially at 70°C, was raised by 20°/min to 90°C, thereafter, the temperature was raised at 5°/min to 140°C, then by 20°/min to 290°C. Injector temperature was raised from 80°C to 280°C at 150°/min and held for 18 min. Detector temperature was set at 280°C. Electron impact ionization, source temperature of 250°C and energy set at 40 eV, was used for mass spectrometry.

Although mass-charge (m/z) ratios for all amino acids were monitored simultaneously, reliable results were achieved only for leucine, isoleucine, valine and alanine. Areas for ion fragments at m/z 158.2 and 159.2, 144.2 and 145.2 and 116.2 and 117.2, corresponding to ^{14}N and ^{15}N ion fragments from leucine and isoleucine, valine and alanine, repectively (Leimer and Gehrke, 1972), were determined using SIM. Run time was 20 min with a dwell time of 50 msec per ion. ^{15}N-enrichment excess in amino acids were determined according to Campbell (1974) using feed, barley and unlabelled digesta and plasma samples to determine natural abundance values.

Statistical Analyses

Where presented, standard error of the means were determined according to principles outlined by Steel and Torrie (1980).

C. Results and Discussion

All barrows consumed their allotted amount of feed, 700 g twice daily, within 1 h of feeding. The animals remained healthy throughout the study. Postmortem examination did not reveal any intestinal abnormalities as a result of cannulation.

The time course of enrichment (% atom excess ^{15}N) in total N in the TCA-soluble and insoluble fractions of plasma and in ileal digesta are illustrated in Figure V-1. The start of the [^{15}N]leucine infusion was marked by a large initial increase in the enrichment in the TCA-soluble fraction of plasma. This initial 'spike' is largely accounted for by ^{15}N in leucine (Figure V-2). A similar spike has not been observed in previous studies with prolonged ^{15}N-leucine infusions (Souffrant et al., 1981; 1993). Enrichments in plasma leucine normally plateau relatively quickly, within 6 h, during continuous intravenous infusions of labelled leucine (Ben-Galim et al., 1980; Haymond and Miles, 1982; Reeds et al., 1992). Furthermore, the incorporation of ^{15}N from infused leucine into other amino acids is equally rapid (Matthews et al., 1979; Ben-Galim et al., 1980). The length of the elevated enrichment in leucine and the lack of a spike in other amino acids raise the possibility that the infusate was also sampled on the 1st d of the [^{15}]leucine infusion. If this were the case, the start of plateau enrichment values in leucine on the 2nd d of the [^{15}N]leucine infusion implies that the position of the catheters had moved in all pigs prior to the 2nd d of the [^{15}]leucine infusion period. Since there is no explained external source for this leucine, this appears to be the only logical conclusion.

With the exception of the initial spike in the TCA-soluble fraction of plasma, enrichments in the two plasma pools followed a similar pattern, with similar enrichments, throughout the infusion period. Neither plasma pool showed any indication of reaching a plateau enrichment, as demonstrated by the extraodinarily high calculated plateau values, 3.23 and 1.76% for the TCA-soluble

and insoluble fractions of plasma, respectively (Table V-2). Enrichments in digesta on d 3 and 8 of the [^{15}N]leucine infusion period were similar to that in the TCA-soluble fraction of plasma.

The lack of a plateau in the enrichment in N in the TCA-soluble fraction of plasma in this study, in contrast to that observed in previous studies (Huisman et al., 1992; Souffrant et al., 1993), is likely related to differences in the protein quality of the experimental diets. Diets used in the previous studies provided a better balance of amino acid compared to barley, thus the consumption of these diets will result in a higher protein turnover rate (Fuller et al., 1987), decreasing the time necessary to achieve a plateau. Furthermore, the higher protein synthesis rate will decrease the oxidation of amino acids (Benevenga et al., 1993) increasing the incorportion of ^{15}N into body proteins. The lack of a plateau in isotopic enrichments are attributed to the recycling of labeled amino acids (Waterlow et al., 1978; Benevenga et al., 1993). Barley in itself does not provide a well balanced protein, particularily with respect to its low lysine content. As a result, protein turnover is expected to be reduced when it is fed as the sole protein source. The slower incorporation of ^{15}N into body proteins will increase the length of the [^{15}]leucine infusion necessary to achieve a plateau. This is reflected in the slow increase in the enrichment in N in the TCA-soluble fraction of plasma (Figure V-1) compared to the initial sharp increase observed in previous studies (Souffrant et al., 1981, 1993).

Similar enrichments in total N in the two plasma pools is in contrast to previous studies with the ^{15}N-isotope dilution technique. In pigs fed diets containing casein or casein with isolated faba bean protein enrichments in the TCA-soluble fraction of plasma were up to 50% higher than that in the TCA-insoluble fraction (Souffrant et al., 1981, 1993). The difference between observations in this study and those in previous studies is a reflection of the different patterns of blood sampling used. Within the current methodology of the ^{15}N-isotope dilution technique enrichments in endogenous N in ileal digesta are assumed to be similar to that in the TCA-soluble fraction of plasma from blood

samples taken at the time of feeding, twice daily (Souffrant et al., 1981, 1993; de Lange et al., 1990, 1992; Huisman et al., 1992). In response to concerns regarding the accuracy of this pattern of blood sampling for estimating the enrichments in endogenous N in ileal digesta over the entire period for which digesta is collected (de Lange et al., 1992), Mosenthin et al. (1993) increased the number of samples to 4/d.

Concentrations of free amino acids increase in plasma following the consumption of a protein-containing meal and remain elevated for several hours (Elia and Livesey, 1983; Rerat, 1985). Recently absorbed dietary amino acids are rapidly incorporated into endogenous proteins (Reeds et al., 1992; Leterme et al., 1993; Souffrant et al., 1993) which may then be secreted into the intestinal lumen. The use of unlabelled dietary amino acids for endogenous protein synthesis will result in a dilution of the enrichment in endogenous N secreted into the intestinal lumen and subsequently recovered from ileal digesta. Reeds et al. (1992) and Souffrant et al. (1993) reported oscillating patterns in the enrichment of endogenous protein that were consistant with periods of dietary absorption. The latter is especially important since it was demonstrated in pancreatic secretions. The enrichment in blood samples taken only at the time of feeding will not reflect this dilution. Digesta samples collected continuously between meals will, however, reflect the different proportions of endogenous protein secreted at different times during the digestive process. An accurate estimate of the enrichment in endogenous protein over the entire period for which digesta is collected is critical for an accurate estimation of the contribution of endogenous to total protein in ileal digesta with the ^{15}N-isotope dilution techniques.

Increasing the frequency of blood sampling is expected to more accurately estimate the enrichment in endogenous protein by taking into account the use of recently absorbed dietary amino acids for endogenous protein synthesis. However, the greater contribution of unlabelled dietary N to total N in the TCA-soluble fraction of pooled plasma (from hourly blood samples) will result in a lower relative enrichment compared to that in plasma from blood samples taken

only at the time of feeding. As a result of the relatively slow turnover rate of plasma proteins, enrichments in the TCA-insoluble fraction of plasma will be less susceptable to short term fluctuations in the plasma free amino acid pool. Plasma proteins have an average turnover rate of approximately 10 d (Wuhrman and Wunderly, 1960) compared to 8 h for the free amino acid pool (Fuerst, 1983). Enrichments in the TCA-soluble and insoluble fractions of pooled plasma are expected to be more similar than that observed in blood samples taken only at the time of feeding.

The pattern of enrichments illustrated in Figure V-1, compared to those presented previously (Souffrant et al., 1981, 1993), suggest that enrichments in N in the TCA-soluble fraction of plasma from blood samples taken only at the time of feeding are overestimated. de Lange et al. (1992) concluded that enrichments in free leucine were also overestimated in blood samples taken only at the time of feeding. An overestimation of the enrichment in the TCA-soluble fraction of plasma will lead to an underestimation of the contribution of endogenous to total protein in ileal digesta, and thus the recovery of endogenous protein. The recovery of endogenous protein in this study was higher, 39.8 (Table V-2) versus 27.7 g/kg DM intake, than that observed previously by de Lange et al. (1990), who also fed a diet containing barley as the sole source of protein.

The implication of this overestimation is disconcerting because it implies that estimates of the contribution of endogenous to total N in ileal digesta, already estimated to be in excess of 80% with various diets (Souffrant et al., 1981, 1993; de Lange et al., 1990), is even higher. The estimated recovery of endogenous N in ileal digesta could, therefore, exceed the actual output of total N in some cases. This raises important questions as to the validy of the ^{15}N-isotope dilution technique used in its current form (Souffrant et al., 1981, 1993; de Lange et al., 1990; Huisman et al., 1992), particularily with respect to the pattern of blood sampling.

In this respect the estimates for endogenous protein recovery at the distal ileum of pigs fed faba bean-casein diets, approximately 17.5 g/kg DM intake

(Mosenthin et al., 1993) are of interest because, not only are they similar to that observed with protein-free diets (e.g., de Lange et al., 1989), but also because they are considerably lower than that observed in this study and that of de Lange et al. (1990). In that study blood samples were only taken every 6 h, compared to every h in this study. The reason for this apparent discrepancy is unclear, but may relate to the relatively slow intestinal absorption rate of legumes which tends to flatten plasma response curves (e.g., Jenkins et al., 1982). A more continuous appearance of dietary amino acids in plasma should provide a more continuous enrichment in plasma and a more continuous incorporation of ^{15}N into endogenous protein. Furthermore, the more continuous appearance of dietary amino acids will minimize differences in metabolism in the fed versus the postabsorptive state which could influence the distribution of ^{15}N in different pools. In effect a constant appearance of dietary amino acids in plasma would be expected to lead to an isotopic equilibrium, not only between feeding periods, but also within feeding periods. The extent to which this contributes to the lower estimates of endogenous recovery deserves further attention. Increasing the frequency of feeding, in conjunction with blood sampling, could only improve estimates of the recovery of endogenous protein because the animals would be in a more stable metabolic and isotopic equilibrium. Estimates of the enrichment in endogenous N, both in ileal digesta and plasma, would be more accurate, making the ^{15}N-isotope dilution technique a more effective method for evaluating factors that affect the recovery of endogenous protein at the distal ileum of pigs fed protein-containing diets.

The time course of enrichments in leucine, isoleucine, valine and alanine are illustrated in Figure V-2. Within each pool the highest enrichments were observed for leucine, followed by isoleucine and valine, then alanine. In agreement with de Lange et al. (1992), enrichments in amino acids were several times higher than that in total N in the different pools. Enrichments in amino acids in the TCA-soluble fraction of plasma were also at least twice that in digesta. Enrichments in digesta exceeded those in the TCA-insoluble fraction of

plasma on d 3 of the [^{15}N]leucine infusion period but were similar on d 8. In contrast to enrichments in total N in the TCA-soluble fraction of plasma and in digesta, plateaus were achieved in amino acids in these pools by the end of the [^{15}N]leucine infusion period (Table V-2). Enrichments in amino acids in the TCA-soluble fraction of plasma, similar that in total N, did not achieve a plateau within the time-frame of this study.

The extent to which the enrichments in amino acids observed in this study reflect the enrichments in endogenous protein secreted into the intestinal is demonstrated by the recent results of Souffrant et al., (1993). The enrichment in total N in pancreatic juice from pigs fed a casein based diet was twice that in the TCA-soluble and insoluble fractions of plasma, 0.38% versus approximately 0.20%. Protein is the predominant source of N in both pancreatic juice (Corring and Jung, 1972) and the TCA-insoluble fraction of plasma and since pancreatic proteins are synthesized using amino acids that are ultimately derived from the plasma free amino acid pool (Simon et al., 1983), these results indicate that the enrichment in plasma free amino acids is approximately twice that in amino acids in plasma proteins. The difference in the enrichments in amino acids in the TCA-soluble and insoluble fractions of plasma were of a similar magnitude. Together, the results of these studies indicate that the ^{15}N-isotope dilution technique, or some version of it, can be an effective tool for estimating the recovery of endogenous protein in ileal digesta.

The results of this study, and that of Souffrant et al. (1993), however, also indicate that the enrichments in total N in the TCA-soluble fraction plasma do not reflect the enrichment in endogenous N secreted into the intestinal lumen. The large differences in the enrichments in free amino acids in the TCA-soluble and insoluble fractions of plasma, despite similar enrichments in total N in these pools, indicate the presence of two distinct sources of N in the TCA-soluble fraction of plasma. Once again this is supported by the large differences in the ^{15}N-enrichment of pancreatic juice versus that in the TCA-soluble fraction of plasma (Souffrant et al., 1993). The first source of N, consisting primarily of free

amino acids, urea and ammonia, is the true precursor pool for endogenous N secretion. Urea and ammonia enter the intestinal lumen directly (Rerat and Buraczewska, 1988) while free amino acids are secreted indirectly via the synthesis of endogenous protein. However, there is also a second pool of N-containing compounds which are apparently not directly associated with the secretion of endogenous N into the intestinal lumen. Collectively, these compounds have a lower enrichment than that in free amino acids, urea and ammonia N combined. The source of this N is unclear, although peptides, nucleic acids, creatinine, amino sugars, etc., are all possibilities.

Results presented in Figure V-2, and in the study by Souffrant et al. (1993) indicate that the nonprecursor pool of N is at least as large as that of the precursor pool. Together, ammonia and urea contribute approximately the same amount of N as amino acids to total ammonia, urea and free amino acid N in plasma (Davey et al., 1973; Chavez and Bayley, 1977). Since the enrichment in urine was similar to that in pancreatic juice, approximately 0.4% (Souffrant et al., 1993), this is approximately the enrichment in the direct precursor pool for endogenous N secretion. However, this was twice that in the TCA-soluble fraction of plasma, indicating, as do the results in Figure V-1, that enrichments in the precursor pool for endogenous N secretion into the intestinal lumen are diluted by this large fraction of nonprecursor N. Enrichments in urine are indicative of enrichments in plasma urea and ammonia because these compounds account for 80 to 95% of the N in urine (Reeds et al., 1980). Enrichments in plasma urea were similar to that in urinary urea following the oral administration of [^{15}N] glycine (Matthews et al., 1981).

Since enrichments in N in ileal digesta reflect that of endogenous N, predominantly in protein, urea and ammonia, dilution of the enrichment in N in the TCA-soluble fraction of plasma by N sources that are not directly associated with the secretion of N into the intestinal lumen will result in an overestimation of the contribution of endogenous to total N in ileal digesta. This is clearly demonstrated in Table V-3. When the enrichment in total N in the TCA-plasma

fraction was considered to reflect the enrichment in endogenous N in ileal digesta, endogenous N was estimated to represent 84.3% of the total N in ileal digesta. However, when the contribution was determined more directly, endogenous leucine, isoleucine, valine and alanine, accounted for an average of 46% of the endogenous leucine, isoleucine, valine and alanine. Assuming this reflects the average recovery of all endogenous amino acids, the maximum contribution of endogenous N would only be around 75%. The maximum contribution of nonamino acid N in ileal digesta in this study was 32.2%. In light of the similar estimates obtained in this study and that of de Lange et al. (1990) for similar barley diets, it is also apparent that the overestimation of the recovery of endogenous N, as a result of dilution in the direct precursor pool, outweighs any underestimation resulting from the pattern of blood sampling. Estimates of the recovery of endogenous N with the ^{15}N-isotope dilution technique will be overestimated if only the enrichment in total N in the TCA-soluble fraction of plasma is considered.

The results presented in this study point out important aspects in the current methodology of the ^{15}N-isotope dilution technique which raise concerns as to the validity of estimates of the contribution of endogenous to total N in ileal digesta of pigs fed protein-containing diets with this technique. Overall, the results support the contention that enrichments in endogenous N need to be determined more directly (de Lange et al., 1992). With respect to the ^{15}N-isotope dilution technique, this may be achieved by more effectively isolating the actual precursor pool for endogenous N secretion, possibly by ultrafiltration or some other means. In addition, alternatives to the ^{15}N-isotope dilution technique, such as the ^{15}N-leucine isotope dilution technique as described by de Lange et al. (1990), or similar techniques, are also worth pursuing. However, the recent results presented by Mosenthin et al. (1993) indicate additional concerns that need to be addressed and suggest that, irrespective of the technique used, some standardization is necessary and that this should include attempts to bring the experimental animals into isotope equilibrium, both between feeding periods and

within feeding periods. Under these conditions, even without isolating the actual precursor pool for endogenous N secretion, the ^{15}N-isotope dilution technique may be a useful tool for evaluating the relative recoveries of endogenous N with different diets.

Table V-1. Composition and dry matter and crude protein content of the diet

Ingredients	%[a]
Barley	90.15
Sucrose	5.00
Canola c	2.00
Dicalciu.. hosphate	0.70
Calcium carbonate	1.10
Mineral premix[b]	0.10
Vitamin premix[b]	0.20
Iodized salt	0.50
Chromic oxide[d]	0.25
Dry matter	91.32
Crude protein	10.37

[a]As fed.
[b]The vitamin premix provide the following (mg/kg diet): retinyl palmitate, 5.2; cholecalciferol, .38; all-rac-α-tocopherol acetate, 44.0; menadione, 2.0; riboflaven, 2.2; niacin, 12.0; d-pantothenic acid, 11.0; vitamin B_{12}, .012; choline, 550; thiamine, 1.1; pyridoxine, 1.1; d-biotin, .1; folic acid, .6.
[c]The mineral premix supplied the following (mg/kg diet): Fe, 50; Zn, 50; Mn, 2; Cu, 3; Se, .15; I, .14.
[d]Fisher Scientific, Fair Lawn, NJ.

Table V-2. Regression equations for plateau ^{15}N-enrichments in N and amino acids in the TCA-soluble and insoluble fractions of plasma[a]

	TCA-soluble	TCA-insoluble
Nitrogen[b]	$y = 3.228(1-e^{-0.001x})$	$y = 1.764(1-e^{-0.003x})$
Leucine[b]	$y = 1.521(1-e^{-0.273x})$	$y = 7.101(1-e^{-0.006x})$
Isoleucine	$y = 0.658(1-e^{-0.216x})$	$y = 14.381(1-e^{-0.001x})$
Valine	$y = 0.649(1-e^{-0.152x})$	$y = 12.941(1-e^{-0.001x})$
Alanine	$y = 0.457(1-e^{-0.117x})$	$y = 3.543(1-e^{-0.002x})$

[a]The regression model was $y = A(1-e^{-bx})$.
[b]Initial spikes in plasma N and leucine (Figures V-1 and V-2) are excluded from these calculations.

Table V-3. The contribution (%) of endogenous N, leucine, isoleucine, valine and alanine to total N, leucine, isoleucine, valine and alanine, respectively, in ileal digesta of pigs fed a barley diet

Item	Contribution
Nitrogen	84.32 ± 8.423[a]
Leucine	36.58 ± 4.305
Isoleucine	45.55 ± 7.673
Valine	51.13 ± 3.510
Alanine	51.54 ± 6.634

[a]Mean ± SEM (n = 3).

Figure V-1. The time course of ^{15}N-enrichment excess in the TCA-soluble (closed circle, dashed line) and insoluble (open circle, solid line) fractions of plasma and in ileal digesta (asterisks) of pigs fed a barley diet and continuously administered [^{15}N]leucine intravenously. Each point represents a 12 h pooled sample (n = 3).

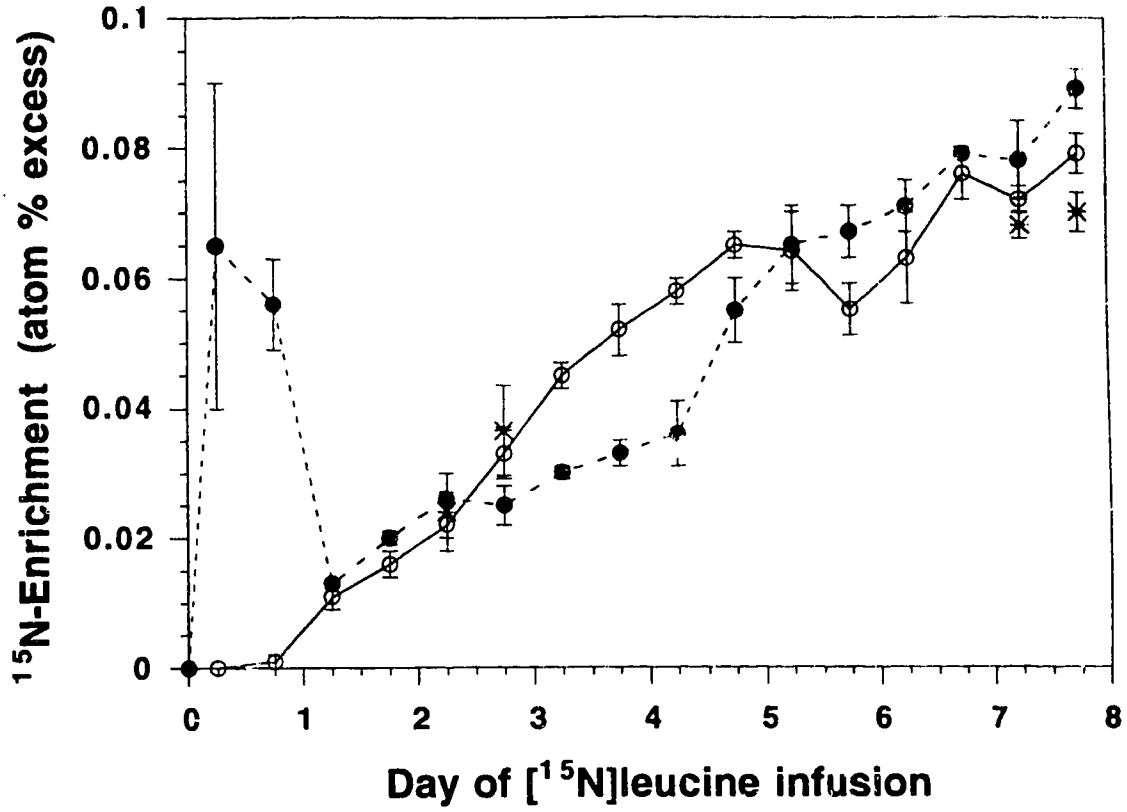
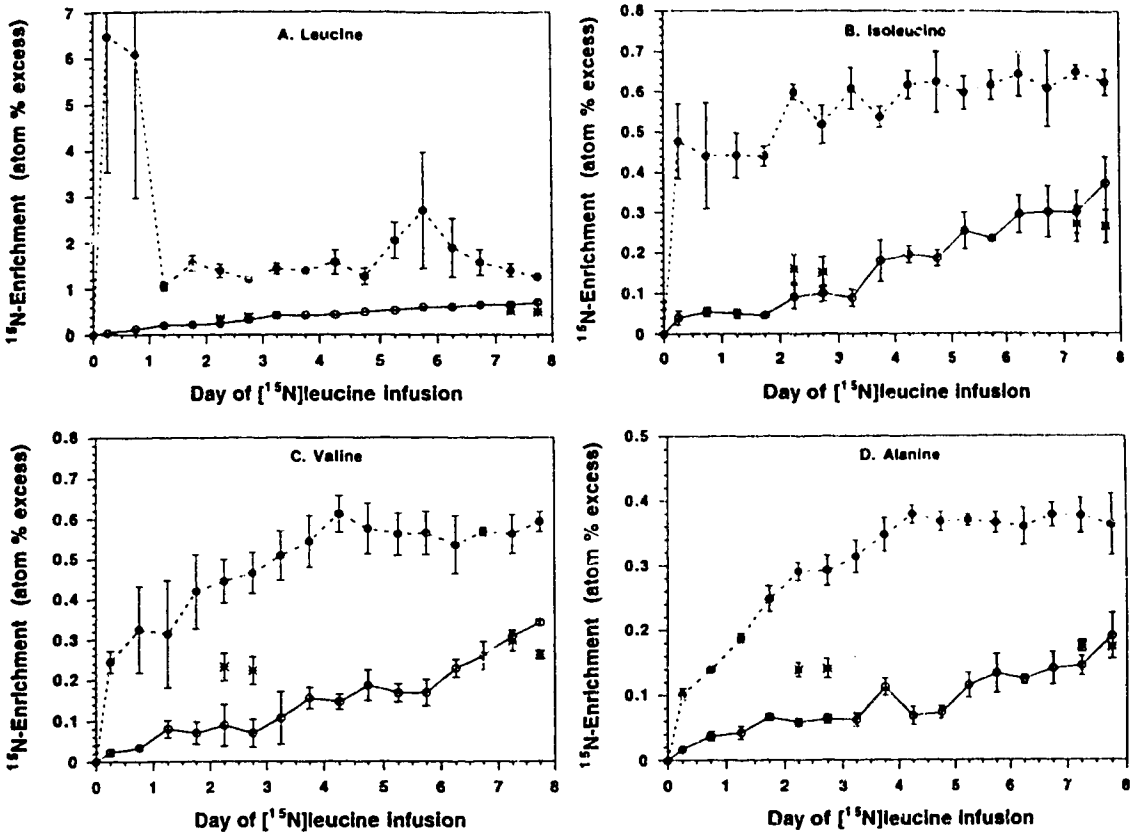

0.1
0.08
0.06
0.04
0.02
0
^{15}N-Enrichment (atom % excess)
0
1
2
3
4
5
6
7
8
Day of [^{15}N]leucine infusion

Figure V-2. The time course of ^{15}N-enrichment excess in a) leucine, b) isoleucine, c) valine and d) alanine in the TCA-soluble (closed circle, dashed line) and insoluble (open circle, solid line) fractions of plasma and in ileal digesta (asterisks) of pigs fed a barley diet and continuously administered [^{15}N]leucine intravenously. Each point represents a 12 h pooled sample (n = 3).


A. Leucine
B. Isoleucine
C. Valine
D. Alanine
^{15}N-Enrichment (atom % excess)
Day of [^{15}N]leucine infusion

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CHAPTER VI

EVALUATION OF THE ^{15}N-ISOTOPE DILUTION TECHNIQUE FOR DETERMINING THE RECOVERY OF ENDOGENOUS PROTEIN IN ILEAL DIGESTA OF PIGS; THE EFFECT OF PATTERN OF BLOOD SAMPLING, PRECURSOR POOLS AND ISOTOPE DILUTION TECHNIQUE

A. Introduction

The ^{15}N-isotope dilution technique is proposed to be a valid method for determining the recovery of endogenous protein at the distal ileum of pigs fed a protein-containing diet (Souffrant et al., 1981, 1986). However, the recovery of endogenous protein is overestimated with this technique (Chapter V) and should be assessed in a more direct manner. In a recent study, de Lange et al. (1992) observed that the recoveries of endogenous protein determined with the ^{15}N-isoleucine and the ^{15}N-isotope dilution techniques were similar while estimates using the ^{15}N-leucine isotope dilution technique were lower than those observed when protein-free diets were fed. It was proposed that methodological considerations with ^{15}N-dilution techniques, especially with respect to the pattern of blood sampling, might explain the results obtained by de Lange et al. (1992).

The purpose of this study was to evaluate the ^{15}N-amino acid dilution technique for determining the recovery of endogenous protein at the distal ileum of pigs. In order to achieve this, some methodological considerations must be addressed. These include the effect of the pattern of blood sampling on the recovery of endogenous protein, a validation of the use of the plasma free amino acid pool to estimate enrichments in endogenous amino acids recovered in ileal digesta and the effect of bacteria on enrichments in endogenous amino acids.

B. Experimental Procedures

Animals and Diets

A detailed description of the experimental procedures and the composition of the experimental diet were presented previously (Chapter V). Three pigs, surgically fitted with a re-entrant cannula at the distal ileum and two catheters in the external jugular veins, were fed twice daily 700 g of a diet containing barley as the sole source of protein. A 1 d intravenous infusion of sterile saline was followed by an 8 d continuous infusion of [^{15}N]leucine (MSD Isotopes, Frosst Merck, Montreal, Que.), via the right jugular catheter. Approximately 30 mg [^{15}N]leucine was infused per kg BW per day. Blood samples were taken hourly beginning with the onset of the saline infusion and continued for 8 d following the initiation of the [^{15}N]leucine infusion. Total collection of digesta was performed hourly on d 3 and 8 of the [^{15}N]leucine infusion period. Twelve and 24 h pooled digesta samples were prepared by combining 30 and 20%, respectively, of each hourly sample. Samples were frozen immediately after each hourly collection. The dry matter, crude protein and amino acid contents of the experimental diet are presented in Table VI-1.

Blood and digesta samples were prepared for analyses as described in Chapter V. Prior to analyses, plasma samples were thawed and pooled over the 12 h periods between feedings by combining 1 mL from each hourly sample. Fifteen percent trichloroacetic acid (TCA) was added and the samples separated into TCA-soluble and insoluble fractions. Digesta samples were weighed, freeze-dried, weighed again, and ground (Model 4 laboratory Wiley Mill, Arthur H. Thomas Co., Philadelphia, PA) through a 1 mm-mesh screen and mixed prior to analysis.

Chemical Analyses

Methods for analyses of CP (N X 6.25), DM, amino acids and chromic

oxide were reported previously (Chapter V). For analyses of amino acid in crude mucin, 30 mg of sample was hydrolyzed in 6 mL 6M HCl to reduce the destruction of serine and threonine. The enrichment (atom % excess ^{15}N) in total nitrogen (N) was determined using an Emission Spectrometer (Isonitromat RFT5201, VEB Statron, Fuerstenwalde, F.R.G.). Soluble crude mucin was isolated from digesta according to procedures outlined in Chapter II. Bacteria in digesta were isolated from the 24 h pooled digesta samples as described by Dugan (1992); bacterial protein and amino acids were quantitated using diaminopimelic acid as a marker.

It was observed after our previous analysis (Chapter V) that sensitivity in the determination of enrichments in amino acids could be improved by better standardization of the amount of individual amino acids, from different samples, injected onto the Gas Chromatography-Mass Spectromy (GC-MS) column. Analysis of the ^{15}N-enrichment excess in amino acids was, therefore, repeated in digesta, crude mucin, bacteria and the pooled TCA-soluble fraction of plasma from d 8 of the [^{15}N]leucine infusion period. As well, the enrichments in amino acids were determined in the TCA-soluble fraction of plasma from blood samples taken at feeding time on d 8 of the [^{15}N]leucine infusion period. Since most of the variation resulted from differences in the recovery of amino acids, from different samples, from the cation exchange column, effluent from the ion exchange cleanup was freeze-dried, redissolved in 1 mL of water and subjected to amino acid analysis. Aliquots containing 10 µg of leucine were then transferred to a second test tube and freeze-dried once again. Plasma samples were heated to convert glutamine to glutamate for comparison to other pools. Trimethylsilyl derivitives were prepared as described previously (Chapter V). The ^{15}N-enrichment excess in all amino acids were determined simultaneously by single ion monitoring GC-MS (Hewlett Packard 5890 Series II gas chromatograph connected to a Hewlett Packard 5971A mass selective detector). Enrichments were determined from the following molecular fragments (PMI/PMI+1 after Campbell, 1974) according to Leimer et al. (1977): alanine (116/117), aspartic acid

(232/233), glutamic acid (246/247), glycine (102/103), isoleucine (158/159), leucine (158/159), phenylalanine (218/219), proline (142/143), serine (204/205), threonine (218/219), tyrosine (218/219) and valine (144/145). The basic amino acids, arginine, histidine and lysine exhibited a poor response on the GC-MS column used in this study. Enrichments were calculated according to procedures outlined by Campbell (1974). Pooled samples of the TCA-soluble fraction of plasma from d 1 of the infusion period (saline infusion) were used to determine natural abundance values for the TCA-soluble fraction of plasma. Barley and the experimental diet (and unlabelled digesta and crude mucin) were used to determine natural abundances for amino acids in digesta, crude mucin and bacteria.

Real ileal amino acid and protein digestibilities were determined as described by Souffrant et al. (1981) and de Lange et al. (1990), by assuming that the ^{15}N-enrichment excess in N and amino acids in the TCA-soluble fraction of plasma was similar to that in endogenous N or amino acids, respectively, in ileal digesta. For the ^{15}N- and ^{15}N-leucine isotope dilution techniques, the composition of endogenous protein was assumed to be similar to that reported for pigs fed a protein-free diet with simultaneous administration of amino acids intravenously (de Lange et al., 1989).

Statistical Analyses

A one-tailed T-test (Steel and Torrie, 1980) was used to assess significance of enrichments. Multiple comparisons were performed using SAS probability of difference (PDIFF) procedures (SAS, 1988) to determine enrichment differences between pools and between different methods of digestibility calculation.

C. Results and Discussion

Enrichments in total N and amino acids in the TCA-soluble fraction of

plasma sampled at the time of feeding and in 12 h pooled samples on d 8 are presented in Table VI-2. Enrichments in total N and amino acids in digesta, crude mucus and bacteria collected at the distal ileum are also presented. The relative enrichments among amino acids in each pool were similar. The highest enrichments were observed in leucine, followed by isoleucine, then valine. The other indispensable amino acids exhibited no significant incorporation of ^{15}N above natural abundance. Enrichments in all of the dispensable amino acids were lower than those in the branched-chain amino acids (BCAA). The highest enrichments in the dispensable amino acids were observed for alanine, aspartate, glutamate and serine; these were lowest for glycine and proline. Similar relative enrichments were observed in plasma amino acids of dogs continuously administered [^{15}N]leucine (Matthews et al., 1979; Ben-Galim et al., 1980). Enrichments in amino acids were up to several times higher than in total N, as was observed previously (Chapter V; de Lange et al., 1992).

The pattern of enrichments among the BCAA, alanine, glutamate and aspartate reflect a well established metabolic relationship between these amino acids. Branched-chain amino acids are primarily metabolized in muscle tissue (Harper et al., 1984). Considerable transamination of leucine (Schwenk et al., 1985a, 1985b) and, since the BCAA aminotransferase has low specificity (Harper et al., 1984), isoleucine and valine occurs in muscle. A rapid transfer of ^{15}N among the BCAA is expected, since under normal circumstances reamination is the primary fate of branched-chain α-keto acids (Harper et al., 1984). Moreover, leucine oxidation increases with elevated levels of leucine (Elia and Livesey, 1983; Cortiella et al., 1988), increasing the transfer of ^{15}N to amino acids such as alanine, glutamine and aspartate which are synthesized in muscle (Harper et al., 1984). This pattern is reflected in the distribution of ^{15}N among amino acids in plasma in this study: leucine 21.3±.54%, alanine 18.1±1.41%, glycine 17.2±1.90%, valine 12.5±1.65%, glutamate 11.7±1.46%, isoleucine 7.5±.75%, serine 4.5±.34%, proline 5.2±.73% and aspartate 2.2±.23% (calculated from enrichments in amino acids and the contribution of amino acids to 100 mg of free amino acid N).

The enrichments in plasma will be a function of the rate of ^{15}N incorporation, the size of the plasma pool and the dietary supply. Incorporation of ^{15}N into amino acids such as glycine, proline and serine will be determined by the amount of *de novo* synthesis and the reamination of their α-keto acids. Similar patterns in the enrichments among amino acids in the different pools suggest that amino acids used for the synthesis of endogenous protein are ultimately derived from the plasma free amino acid pool.

The relative enrichments in amino acids and N in the various pools raise important implications for the use of ^{15}N-isotope dilution techniques for assessing the contribution of endogenous to total protein in ileal digesta of pigs fed protein-containing diets. Traditional ^{15}N-isotope dilution techniques rely on plasma samples taken at feeding times (usually every 12 h) to approximate the enrichment in endogenous protein secreted into the intestinal lumen (Souffrant et al., 1981; de Lange et al., 1990). However, endogenous protein synthesis and secretion is a continuous process and the enrichment in endogenous protein in digesta will reflect the synthesis and secretion of endogenous protein over the entire 12 h period between feedings. There is some question, therefore, as to whether blood samples taken at time of feeding accurately relect the enrichment in endogenous amino acids in ileal digesta (de Lange et al., 1992).

While the majority of the amino acids exhibited no difference ($P > .20$) in enrichments between pooled plasma samples and those taken at feeding, enrichments in leucine and alanine were 40% and 13% higher ($P < .02$), respectively, in plasma samples taken at feeding time (Table VI-2). Reeds et al. (1992) observed lower D_3-enrichments in leucine, alanine, phenylalanine and lysine in plasma of humans in the absorptive state, compared to those in a postabsorptive state. Dilution by dietary amino acids was the most obvious reason. A similar pattern was observed in VLDL apolipoprotein B-100, a plasma protein with a relatively rapid turnover rate. These observations indicate that the enrichments in endogenous proteins, will follow those observed in plasma. Since the enrichments in amino acids in pooled plasma samples are expected to better

reflect the enrichments in endogenous amino acids in digesta over the entire period which digesta is collected, estimates of the enrichments in endogenous leucine and alanine are overestimated in blood samples taken at feeding time. An overestimation of the enrichments in endogenous amino acids will lead to an underestimation of their endogenous recoveries in ileal digesta (Chapter V). This explains the lower recovery of endogenous protein and amino acids observed by de Lange et al. (1992) using the ^{15}N-leucine isotope dilution technique compared to the ^{15}N and ^{15}N-isoleucine isotope dilution techniques.

The enrichments in leucine and the other amino acids is dependent upon the rate of catabolism of labelled leucine and thus the distribution of ^{15}N among amino acids. Leucine catabolism is largely controlled by its plasma levels (Castellino et al., 1987; Motil et al., 1994), with higher transamination and oxidation rates in the absortive, compared to the postabsorptive state (Elia and Livesey, 1983; Cortiella et al., 1988; Motil et al., 1994). Moreover, the quality of protein consumed, i.e. the extent to which the amino acid composition matches that required for protein synthesis, can also influence the catabolism of amino acids (Fuller et al., 1987; Benevenga et al., 1993). Thus, in addition to dilution, enrichments in leucine may be elevated in blood samples taken at time of feeding (postabsorptive state) because of its lower rate of catabolism. The extent to which a postabsortive state is achieved, and thus the influence on the enrichments in leucine and other amino acids will be dependent on the rates of digestion, absorption and metabolism of different protein sources, as opposed to simply providing an accurate estimate of enrichments in endogenous amino acids. An accurate estimate of the enrichment in endogenous amino acids in digesta can only be achieved by frequent blood sampling which takes into account changes in enrichments throughout the entire period between feedings.

The higher enrichments (27%; P = .13) in glutamate in plasma samples obtained at time of feeding, compared to pooled samples, is primarily the result of dilution. The high contribution of glutamate to glutamate plus glutamine (30 to 40%) in pooled plasma samples in this study is due to the presence of dietary

glutamate, since the contribution of glutamate in the postabsorptive state is considerably lower (Elia and Livesey, 1983; Castellino et al., 1987). This is supported by the fact that the highest enrichments in glutamate were observed in crude mucin, since the intestine is the principal destination for glutamine synthesized in muscle (Souba et al., 1990). An increase in the synthesis of glutamine in the postabsorptive state (prior to feeding), in conjunction with the oxidation of leucine, will also elevate its enrichment in plasma samples taken at the time of feeding.

Another consideration in the methodology of ^{15}N-isotope dilution techniques is whether the enrichments in plasma amino acids are valid estimates of the enrichments in endogenous amino acids in digesta. The plasma free amino acids represent the precursor pool for pancreatic protein synthesis (Simon et al., 1983) and these proteins exhibit rapid incorporation of intravenous (Simon et al., 1983) and orally administered labels (Leterme et al., 1994). Therefore, enrichments in plasma amino acids might be expected to provide a reliable estimate of the labelling in these and other proteins, e.g. salivary proteins. However, the intestinal mucosa is the single largest contributor to endogenous protein in the intestinal lumen (Souffrant et al., 1986). The precursor pool for mucosal protein synthesis is, as yet, unidentified, although it has been shown to use lumenal amino acids (Hirscfield and Kern, 1969; Alpers, 1972). The use of lumenal amino acids for endogenous protein synthesis in the intestinal mucosa could result in a dilution of ^{15}N in endogenous amino acids that would not be reflected in the enrichments in plasma amino acids (Souffrant et al., 1986). In this situation the enrichments in plasma amino acids would overestimate the actual enrichment in endogenous amino acids, leading to an underestimation of their recovery in digesta. This is doubly important if a considerable proportion of unlabelled dietary amino acids are employed for intestinal protein synthesis, and the protein is relatively indigestible. In this respect, the enrichments in mucus are of importance since it is not digested in the small intestine (Chapter II; Hoskins, 1984).

Threonine, serine and proline represented 32.5±1.13 mol/100 mol amino acids in crude mucin (Table VI-3) compared to 40 mol/100 mol in crude mucin from pigs fed a protein-free diet (Chapter II) and 50 to 70 mol/100 mol amino acids in purified mucus (Allen, 1981). These values demonstrate that while mucus represents a large portion of the protein in this fraction of digesta, the majority is nonmucin protein. Similar enrichments in the BCAA, and higher enrichments in glutamate, in crude mucin compared to pooled plasma samples (Table VI-2) suggest that the contaminant protein is predominantly endogenous in origin. The higher enrichment in glutamate is important, since the intestine is a primary destination for glutamine synthesized in conjunction with the catabolism of leucine (Souba et al., 1990). Lower ($P < .05$) enrichments in alanine and glycine may reflect the *de novo* synthesis of these amino acids. Moreover, enrichments in amino acids in crude mucin were up to 2.6 times higher than their counterparts in digesta, further demonstrating that amino acids in crude mucin were predominantly of endogenous origin. The nonmucus protein may be noncovalently bound endogenous protein (Allen, 1981) or soluble endogenous protein coprecipitating with crude mucin. That the amino acids in crude mucin are apparently endogenous, and that their enrichments were similar to those in pooled plasma samples, supports the use of enrichments in free amino acids in the TCA-soluble fraction of pooled plasma samples to estimate enrichments in endogenous protein recovered at the distal ileum.

Bacteria in digesta present further potential complications for the use of ^{15}N-isotope dilution techniques. Bacteria represented 27.4% of the N in ileal digesta (Table VI-3). The higest contributions were for methionine (49.4%), lysine (37.6%) and isoleucine (30.8) while the lowest contributions were for proline (11.5%) and glycine (17.8%). These values are in agreement with previous estimates (Dierick et al., 1983; Drochner, 1984; Dugan, 1992). Furthermore, bacteria possess the ability to use nonprotein N for the *de novo* synthesis of amino acids. Nonprotein ^{15}N is not uniformly incorporated into all bacterial amino acids (Takahashi et al., 1980; Takahashi and Kametaka, 1986). Bacteria, therefore, have

the potential to modify the amino acid composition of digesta (Dugan, 1992) and in doing so, alter enrichments in endogenous amino acids which may not be accurately reflected in the enrichments in plasma amino acids. There were no differences between enrichments in N ($P = .14$) and amino acids ($P > .30$) in digesta and bacteria (Table VI-2). In addition, the contribution of bacterial amino acids to endogenous and digesta amino acids was similar (Table VI-3). Therefore, bacteria did not alter the labelling of endogenous protein, indicating that there was little de novo synthesis of amino acids by bacteria and that they did not preferentially use either endogenous or unabsorbed dietary amino acids for protein synthesis.

The contributions (% of digesta protein and amino acids) and recoveries (g/kg DM intake) of endogenous protein and amino acids, calculated with three different dilution techniques, are presented in Tables VI-4 and VI-5, respectively. Since these values represent different calculations of the same data, patterns and statistical differences are essentially the same and will be discussed as such. With the exceptions of glutamate and proline, the highest contributions and recoveries of endogenous protein and amino acids were observed with the ^{15}N-isotope dilution technique. Endogenous amino acids were overestimated to account for nearly all amino acids in ileal digesta with this technique and were at least twice that observed with the other techniques. Values obtained with the ^{15}N-isotope dilution technique are overestimated (Chapter V). The contribution and recovery of proline determined with the ^{15}N-amino acid dilution technique were approximately 2 and 4 times higher ($P < .05$) than observed with the ^{15}N- and ^{15}N-leucine isotope dilution techniques, respectively. Estimates for glutamate with the ^{15}N-leucine isotope dilution technique were only one-half that observed with the other techniques. Contributions and recoveries of endogenous amino acids were usually higher when determined with the ^{15}N-amino acid dilution technique compared to the ^{15}N-leucine dilution technique. A comparison of the recoveries of amino acids estimated with the ^{15}N-amino acid and ^{15}N-leucine isotope dilutions techniques (Table VI-5) illustrates that there is considerable modification

of the composition of endogenous protein when a barley diet is fed compared to that observed with a protein-free diet. The recoveries of valine, alanine, glutamate and proline were higher with ^{15}N-amino acid dilution technique and thus represent a greater proportion of endogenous protein with the barley diet.

One point worth noting in these data is that the recovery of endogenous proline (5.49±1.73 g/kg DMI) estimated with the ^{15}N-amino acid dilution technique was similar to that observed in pigs fed protein-free diets without amino acid infusion (de Lange et al., 1989). This observation suggests that dietary residuals in the intestinal lumen interfere with the digestion of endogenous protein (Percival and Schneeman, 1979) since an improvement in the protein status of animals (in this case by feeding a protein-containing diet) is proposed to improve the digestion and absorption of endogenous protein (Chapter II; de Lange et al., 1989). The relatively low digestibility of proline in barley, determined with the ^{15}N- and ^{15}N-leucine isotope dilution techniques, in this study (Table VI-5) and in previous studies by de Lange et al., (1990, 1992), is attributed to the presence of endogenous proline.

The apparent and real ileal protein and amino acid digestibilities are presented in Table VI-6. The apparent digestibilities were lower than previously observed for barley (Sauer and Ozimek, 1986; de Lange et al., 1990). There are no differences in ileal digestibilities in pigs fitted with re-entrant versus T-cannulas (Sauer and Ozimek, 1986). However, the fact that particular attention was given to collecting all digesta in this study may have resulted in the collection of relatively more endogenous protein, accounting for the lower digestibilities. Similar real digestibilities in this study and the study by de Lange et al. (1990) gives some support to this.

The highest amino acid digestibilities were observed with the ^{15}N-isotope dilution technique. Real digestibilities of most amino acids were higher when determined with the ^{15}N-amino acid versus the ^{15}N-leucine isotope dilution technique, although this was significant for glutamate, glycine and proline only. Ileal digestibilities of glutamate and proline determined with the ^{15}N-amino acid

dilution technique were not different ($P > .05$) from those observed with the ^{15}N-isotope dilution technique, although the average proline digestibility was 46% higher with the former technique. Glycine digestibilities were different ($P < .05$) with all techniques. These results demonstrate that not only is the ^{15}N-amino acid dilution technique more sensitive to different levels of endogenous protein (versus the ^{15}N-isotope dilution technique), it is also more useful in detecting the outputs of different endogenous proteins (versus the ^{15}N-leucine isotope dilution technique).

In this, and a previous study (Chapter V), an evaluation of ^{15}N-isotope dilution techniques for determining the recovery of endogenous protein at the distal ileum of pigs fed protein-containing diets was presented. Despite the advantages of the ^{15}N-amino acid dilution technique there are important considerations that must still be addressed. Several of the indispensable amino acids did not exhibit any incorporation of ^{15}N and thus estimates of their endogenous recoveries could not be obtained. Determining the recoveries of these amino acids would require that a mixture of labelled amino acids be infused, however, given the cost of labelled amino acids the best alternative may be the use of the ^{15}N-leucine isotope dilution technique. With this technique, assessment of the recovery of endogenous amino acids could be made more feasable by allowing a smaller quantity of [^{15}N]leucine to be infused since only the enrichments in leucine would be required.

A second consideration is with respect to the pattern of blood sampling. In this study each hourly blood sample was given equal weight in assessing the enrichment in endogenous amino acids. However, enrichments in plasma are higher in the postabsorptive period when there is less synthesis of endogenous protein, compared to the absorptive period when endogenous protein synthesis is expected to be at a maximum. Thus the importance of enrichments in plasma amino acids in the absorptive and postabsorptive periods are under- and overemphasized, respectively. Increasing the frequency of meals may rectify this by reducing or eliminating the postabsorptive period through a more constant

passage of digesta, secretion of endogenous protein and absorption of amino acids. Alternatively, enrichments could be determined in a representative endogenous protein, such as purified mucin, which would reflect the overall synthesis and secretion of endogenous protein in reponse to a meal.

Table VI-1. Dry matter, crude protein and amino acid content of the experimental diet

Item	%[a]
Dry Matter	91.3
Crude Protein	11.4
Indispensable Amino Acid	
Arginine	0.46
Histidine	0.23
Isoleucine	0.37
Leucine	0.71
Lysine	0.43
Methionine	0.12
Phenylalanine	0.57
Threonine	0.38
Valine	0.57
Dispensable Amino Acids	
Alanine	0.46
Aspartic Acid	0.62
Glutamic Acid	2.83
Glycine	0.46
Proline	1.16
Serine	0.18
Tyrosine	0.23

[a]as fed basis.

Table VI-2. The ^{15}N-enrichment (atom % excess) in total N and amino acids in the trichloroacetic acid soluble fraction of plasma and in ileal digesta, crude mucin and bacteria on d 8 of the [^{15}N]leucine infusion period

Items	^{15}N-Enrichment[a]:				
	Plasma (pooled)[b]	Plasma (feeding)[c]	Digesta	Crude Mucin[d]	Bacteria[d]
Nitrogen	.08±.002[f]	ND[e]	.07±.004[f]	ND	.07[g]
Indispensable amino acids					
Isoleucine	.75±.044[f]	.81±.064[f]	.28±.037[g]	.74±.031[f]	.25[g]
Leucine	1.37±.012[f]	1.89±.228[g]	.52±.027[h]	1.04±.016[f]	.50[h]
Phenylalanine	-.05±.210	-.04±.048	-.15±.009	-.19±.011	-.21
Threonine	-.01±.023	.05±.030	-.00±.004	-.06±.006	-.05
Valine	.54±.048[f]	.56±.041[f]	.30±.008[g]	.55±.036[f]	.30[g]
Dispensable amino acids					
Alanine	.40±.002[f]	.45±.021[g]	.18±.012[h]	.33±.024[i]	.18[h]
Aspartate	.48±.075[fh]	.51±.056[f]	.26±.013[gi]	.37±.024[gh]	.22[i]
Glutamate	.26±.036[fg]	.33±.019[gi]	.16±.008[fh]	.42±.035[i]	.17[f]
Glycine	.27±.036[f]	.24±.018[fg]	.17±.036[gh]	.15±.007[h]	.14[h]
Proline	.13±.017	.08±.053	.11±.002	.15±.021	.15
Serine	.39±.017[f]	.37±.015[f]	.17±.022[g]	.32±.059[f]	.10[g]
Tyrosine	.05±.052	.04±.101	.04±.026	.10±.020	-.06

[a]Mean ± SEM; n = 3 for all pools except bacteria (n = 2). All enrichments are different ($P < .05$) from 0 using a one-tailed T test, except proline in plasma at feeding time, all phenylalanine and threonine and all tyrosine except that in crude mucin.

[b]Plasma (pooled): plasma samples collected hourly and pooled over 12 h.

[c]Plasma (feeding): plasma samples collected at time of feeding (every 12 h).

[d]Crude mucin and bacteria from ileal digesta.

[e]ND: not determined.

[fghi]Values in same row with different superscripts differ ($P < .05$).

Table VI-3. The amino acid composition (% of total amino acids) of crude mucin and bacteria in ileal digesta of pigs fed a barley diet and the contribution (%) of bacteria crude protein and amino acids to digesta and endogenous crude protein and amino acids, respectively

Item	Crude Mucin mol/100mol[a]	Bacteria		
		mg/100mg	Digesta	Endogenous[b]
Crude Protein			27.4	25.3
Indispensable amino acids				
Arginine	3.4 ± .14	5.2	26.8	
Histidine	2.0 ± .06	2.2	22.2	
Isoleucine	4.5 ± .11	4.6	30.8	32.7
Leucine	6.8 ± .05	6.6	25.7	25.7
Lysine	5.6 ± .27	7.9	37.6	
Methionine	1.1 ± .10	1.6	49.4	
Phenylalanine	3.2 ± .04	4.7	25.7	
Threonine	12.9 ± .56	5.4	25.4	
Valine	7.3 ± .13	5.7	25.1	25.7
Dispensable amino acids				
Alanine	8.6 ± .35	7.9	29.8	29.4
Aspartate	10.1 ± .09	9.7	28.7	23.0
Glutamate	9.8 ± .06	13.5	20.1	21.7
Glycine	11.7 ± .35	6.6	17.8	13.9
Proline	9.5 ± .99	10.5	11.5	16.4
Serine	10.2 ± .31	4.4	21.2	13.4
Tyrosine	2.8 ± .14	3.3	28.3	

[a]Mean ± SEM (n = 3). For bacteria n = 2.
[b]Contribution of amino acid ^{15}N in bacteria to amino acid ^{15}N in digesta.

Table VI-4. The contribution (%) of endogenous protein and amino acids to total protein and amino acids, respectively, in ileal digesta of pigs determined with different isotope dilution techniques

Item	$^{15}N^{a}$	^{15}N-Amino acids[b]	^{15}N-Leucine[a]
Crude Protein	84.3 ± 8.42[c]		351 ± 237[d]
Indispensable amino acids			
Arginine	79.4 ± 3.39[c]		341 ± 574[d]
Histidine	72.5 ± 7.12[c]		302 ± 208[d]
Isoleucine	99.3 ± 15.36[c]	37.2 ± 6.40[d]	407 ± 160[d]
Leucine	92.4 ± 12.31[c]	38.1 ± 1.63[d]	381 ± 168[d]
Lysine	82.3 ± 16.32		332 ± 224
Methionine	105.0 ± 13.42[c]		433 ± 175[d]
Phenylalanine	88.9 ± 10.43[c]		369 ± 214[d]
Threonine	133.7 ± 20.18		548 ± 188
Valine	92.3 ± 13.72[c]	56.4 ± 6.53[d]	379 ± 190[e]
Dispensable amino acids			
Alanine	86.2 ± 13.67[c]	44.8 ± 2.92[d]	353 ± 182[e]
Aspartate	112.2 ± 19.79[c]	55.5 ± 6.37[d]	457 ± 226[d]
Glutamate	67.6 ± 8.38[c]	62.0 ± 8.48[c]	280 ± 145[d]
Glycine	90.7 ± 4.01[c]	62.6 ± 4.81[cd]	385 ± 479[d]
Proline	42.3 ± 13.54[c]	83.3 ±12.36[d]	199 ± 937[e]
Serine	113.0 ± 15.35[c]	44.5 ± 3.56[d]	465 ± 178[d]
Tyrosine	141.0 ± 30.09		566 ± 467

[a]Calculated with the ^{15}N- and ^{15}N-leucine isotope dilution techniques according to de Lange et al. (1990, 1992) using enrichments in N and amino acids in pooled plasma samples to estimate enrichments in endogenous protein and amino acids, respectively.

[b]Calculated from the dilution in individual amino acids using enrichments in amino acids in pooled plasma samples to estimate enrichments in endogenous amino acids.

[c-e]Values in same row with different superscripts differ ($P < .05$).

Table VI-5. Recoveries (g/kg DMI) of endogenous protein and amino acids at the distal ileum of pigs determined with different isotope dilution techniques

Technique	^{15}N[a]	^{15}N-Amino acids[a]	^{15}N-Leucine[a]
Crude Protein	39.82 ±5.30[b]		16.64 ±1.93[c]
Indispensable amino acids			
Arginine	1.24 ± .16[b]		.52 ± .06[c]
Histidine	.47 ± .06[b]		.20 ± .02[c]
Isoleucine	1.33 ± .18[b]	.52 ± .13[c]	.56 ± .06[c]
Leucine	2.08 ± .28[b]	.87 ± .10[c]	.87 ± .10[c]
Lysine	1.60 ± .21[b]		.67 ± .08[c]
Methionine	.29 ± .04[b]		.12 ± .01[c]
Phenylalanine	1.42 ± .19[b]		.59 ± .07[c]
Threonine	2.58 ± .34[b]		1.08 ± .12[c]
Valine	1.86 ± .25[b]	1.17 ± .22[c]	.78 ± .09[d]
Dispensable amino acids			
Alanine	2.05 ± .27[b]	1.08 ± .12[c]	.86 ± .10[d]
Aspartate	3.40 ± .45[b]	1.69 ± .04[c]	1.42 ± .16[c]
Glutamate	2.93 ± .52[b]	3.65 ± .69[b]	1.64 ± .19[c]
Glycine	2.84 ± .38[b]	1.97 ± .33[bc]	1.18 ± .14[c]
Proline	2.29 ± .30[bc]	5.49 ±1.73[b]	.96 ± .11[c]
Serine	2.10 ± .28[b]	.84 ± .13[c]	.88 ± .10[c]
Tyrosine	1.56 ± .21[b]		.65 ± .08[c]

[a]Refer to Table 4.
[bcd]Values in same row with different superscripts differ ($P < .05$).

Table VI-6. Apparent and real ileal crude protein and amino acid digestibilities (%) in barley determined with different isotope dilution techniques

Item	Apparent	Real ^{15}N[a]	Real ^{15}N-Amino Acid[a]	Real ^{15}N-Leucine[a]
Crude Protein	58.5± 2.60	93.6± 3.65		73.2± 1.34
Indispensable amino acids				
Arginine	66.7± 3.45	93.3± .91		77.8± 3.75
Histidine	71.6± 1.78	92.2± 2.09		80.2± 1.03
Isoleucine	63.1± 3.40	99.2± 6.03[b]	77.1± 1.77	78.2± 1.77
Leucine	67.7± 2.44	97.3± 4.30[b]	80.1± 1.01	80.1± 1.01
Lysine	52.7± 6.58	90.0± 9.33		68.3± 5.06
Methionine	76.6± 1.81	101.0± 3.32		86.8± .63
Phenylalanine	71.7± 1.74	96.8± 3.13		82.2± .60
Threonine	49.7± 4.72	115.9±10.02		77.4± 1.71
Valine	63.1± 2.79	96.8± 5.39	84.3± 1.63	77.2± 1.28
Dispensable amino acids				
Alanine	47.8± 4.35	92.1± 7.82[b]	71.2± 2.69	66.3± 2.44
Aspartate	48.2± 5.87	104.8±10.44[b]	76.2± 6.03	71.8± 3.51
Glutamate	78.6± 1.44	93.0± 2.05	92.0± 1.70	84.6± .74[c]
Glycine	32.7± 5.88	94.2± 2.41[b]	75.4± 1.25	58.4± 5.75[c]
Proline	40.9±21.64	60.8±19.29	88.8±10.22	49.2±21.90[c]
Serine	60.2± 3.20	104.8± 6.34[b]	78.1± 1.14	78.9± 1.06
Tyrosine	48.8± 9.31	116.4±14.45		77.1± 6.52

[a]Refer to Table 4.
[c]Different ($P < .05$) from real ^{15}N-amino acid digestibilities.
[d]Different ($P < .05$) from real ^{15}N-amino acid digestibilities.

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CHAPTER VII

GENERAL DISCUSSIONS AND CONCLUSIONS

Interest in the output of mucin at the distal ileum is sparked by three separate, but related, observations. Firstly, in most studies only the digestibilities of glycine and proline are typically lower than that of threonine at the distal ileum (Sauer and Ozimek; 1986). In addition, the disappearance of threonine in the large intestine is among the highest of amino acids. Secondly, threonine is typically the most abundant indispensable amino acid in ileal digesta from pigs fed protein-free diets (e.g., de Lange et al., 1989). Mucus, because of its high threonine content has been implicated, although never demonstrated, as the primary source of this amino acid in endogenous protein. The presence of mucin in ileal digesta is proposed, therefore, to account for the low digestibility of threonine in many feedstuffs. Such a proposition does not seem unreasonable considering that mucus provides a protective lining for the entire gastrointestinal tract and is, therefore, exposed to all of the chemical and physical forces of digestion (Allen, 1981). The third observation, that there is little apparent digestion of mucin prior to the large intestine (Hoskins, 1984) where it is extensively fermented by gut microbes, provides a basis for the first two observations. Together these observations suggest that mucin could represent a considerable proportion of endogenous protein entering the large intestine. Determination of the amount of mucin in ileal would further our understanding of the effects of diets on the digestive process and would aid in assessing the true value dietary protein sources. This is especially important for threonine since it is considered a limiting amino acid for many feedstuffs.

A. Measurement of Mucin

Several techniques have been used to assess the content of mucin in various areas of the digestive tract in response to different stimuli. These include histochemical staining, lectin binding assays, immunnological techniques (radioimmunoassays and ELISA), incorporation of isotopes and purification (Mantle and Allen, 1989: Reid and Park, 1990). However, the methodology of these techniques appears to make them unsuitable for use in determining the content of mucin in ileal digesta following the consumption of a meal. In addition, many of these techniques are based on qualitative measurements of mucus as opposed to quantitative measurements.

Lectin binding assays have been developed to take advantage the affinity of lectins to specific sugar residues in mucin, for determining relative contents of this glycoprotein. This technique is not suitable for determining the contents of mucin in ileal digesta for two reasons. Since lectins show affinity towards specific carbohydrates, i.e., galactose, fucose or amino sugars, the contribution of the sugars from other sources, including the diet will interfer with an accurate measurement. The presence of galactose and fucose containing polysaccharides in the diet would therefore, require the use of lectins expressing affinity toward either of the amino sugars since these are not commonly found in typical feedstuffs. However, these lectins have also been demonstrated to bind with bacteria also (Hughes, 1976). In addition, bateria themselves can bind to mucins (Neutra and Forstner, 1987). Secondly, the presence of lectins in feedstuffs for swine, including some of the most common feedstuffs such cereals (Marsh, 1992), soybeans and especially legumes (Begbie and Pusztai, 1989; Jauregui et al., 1991), and the fact that lectins survive digestion in the gastrointestinal tract (Begbie and Pusztai, 1989), is most likely to desensitize this technique for measuring mucin in ileal digesta. Frier et al. (1985) have presented an assay involving hog gastric mucin in conjunction with ovomucoid, a trypsin inhibitor, for the isolation of

lectins. With this method they isolated 30 lectins from 27 different plants.

The use of immunoassays to quantitate mucin excreted in ileal digesta has some appeal since there are antibodies that are more specifically directed towards mucin, either towards the carbohydrates that confer blood group activities or other areas of the molecule (Reid and Park, 1990). Since little digestion of mucin carbohydrates occurs prior to the large intestine (Hoskins, 1984), antibodies directed towards blood group carbohydrates may be useful. However, the use of antibodies directed towards other areas of the mucin molecule may be less effective. Mantle et al. (1984a, 1986) have demonstrated that the major antigenic determinant of gastrointestinal mucin is associated with the nonglycosylated, naked, region of the mucin molecule and requires that the disulphide bridges be intact. These regions of mucin are susceptable to proteolytic digestion (see Chapter I) and this action reduces the antigenicity of mucin molecules (Mantle et al., 1984a, 1986). More recently, antibodies directed at the 'link' region of mucin molecules have been used, however, this region is also liberated by proteolysis (Mantle et al., 1984b; Fahim et al., 1987). In addition, nonmucin substances, even in highly purified samples (Rabel et al., 1994), may interfere with antigenic reactions.

An obvious alternative to these methods is to quantitate mucin in ileal digesta by isolation and purification. However, this is easier said than done. In the studies carried out for this thesis it was observed, similar to Clamp and Gough (1991), that a significant proportion of the GalNAc in ileal digesta is in an insoluble form, 20 to 40%. Thus, although soluble mucin could be purified it does not provide a representation of the total amount of mucin in ileal digesta. This was demonstrated in Chapter II. The carbohydrate composition of crude mucin recovered from ileal digesta of pigs fed a protein-free diet was typical of gastrointestinal mucins, the presence of which could account for essentially all of the carbohydrates in this fraction of digesta. As expected mucin was demonstrated to consist of a mixture of gastric and intestinal mucin. With respect to estimating the output of mucin in ileal digesta, there may be little value in

purifying the soluble mucin, especially considering the multiple origins. However, the composition of purified gastric and intestinal mucin from the test pigs would most likely provide a more accurate estimate of the content and source of mucin in ileal digesta. In addition, soluble mucin was 24% higher in amino acid infused pigs, however, overall output was estimated to be only 6% higher. Therefore, methods which cannot measure both soluble and insoluble mucin may not provide an accurate reflection of treatment effects.

Many techniques for measuring mucin contents in samples, particularly that of purification (Allen, 1981), involve long laborious processes that are not feasible for use in nutrition studies where large numbers of samples are obtained. It is, therefore, useful to have a rapid and convenient technique for estimating mucin output. The method that I have adopted for the studies presented in this thesis appears to serve this purpose. The usefulness of this method relies on the presence of amino sugars, especially GalNAc, from other sources. N-Acetylgalactosamine is considered to be a useful marker for mucin contents because it occurs in only a limited number of substances, including certain proteoglycans, glycolipids, glycoproteins and bacterial cell walls. The absence or low contents of these compounds is expressed by the levels of other indicator carbohydrates. Rhamnose and ribose, which represent a considerable portion of bacterial cell walls (Walla et al., 1984; Gilbert et al., 1988) were presence in only trace amounts in digesta from pigs fed a protein-free diet (Chapter II). The higher daily outputs of ribose in human ileostomy effluent (Chapter III) and of ribose and rhamnose in digesta from pigs fed increasing levels of pea fiber are attributed to their presence in nonstarch polysacchaides (NSP) in the diets. Thus despite accounting for 20 to 30% of the protein in ileal digesta from pigs (Chapter VI), bacteria contribute only trace amounts of carbohydrates. This is made even more obvious by the fact that amino sugars, galactosamine, glucosamine and muramic acid represent only 1.1% of fecal dry matter, of which most is expected to be bacteria, from humans fed a low-fiber diet (Kraus et al., 1990). Clamp and Gough (1991) observed only trace amounts of GalNAc and GlcNAc in feces from

humans.

The contribution of glycoproteins from sources other than the mucus layer, if present, may affect estimates of mucin output in ileal digesta. While these mucins most certainly enter the digestive, with saliva, pancreatic juice, bile and as a component of shed epithelial cells, their quantities and fate are yet to be determined. However, given the relative size of these sources compared to that of the mucus layer, and the proximity of the mucus layer to the digestive processes the contribution from these other sources may be expected to be small.

The presence or absence of proteoglycans would be indicated by the uronic acid content of digesta. Proteoglycans are comprised of repeating disaccharides consisting of uronic acid and an amino sugar, of which GlcNAc is more common (Roden, 1980). Only 0.1 g of uronic acid was excreted in ileostomy effluent from humans fed an NSP-free diet (Englyst and Cummings, 1986). This was similar to the amount of GlcNAc in ileostomy effluent which was not accounted for by mucin, 0.09 and 0.13 g/day in females and males, respectively (Table III-6). This is of interest since it suggests that, next to mucins, proteoglycans might be the primary source of amino sugars in ileal digesta. In addition, it does tend to confirm observations that the consumption of soy fiber did not affect the intestine, as determined by a lack of effect on mucin output (Chapter III) and provides further evidence for a connection between physical abrasion and the amount of mucin entering the large intestine. In pigs (Chapters II and IV) the estimated GlcNAc output in mucin represented considerably less of the total amount of GlcNAc in ileal digesta than that in humans, 50 to 60% versus 87%. A higher output of proteoglycans might suggest greater damage to the gastrointestinal mucosa, since this is a primary source of these substances, especially from the lamina propria. If this were the case, it could be attributed to the relative amounts of bulk moving through the digestive tract (Chapter III).

With the exception of immunoassays and purification, estimation of the mucin content of ileal digesta by measuring the amino sugar is on par with many of the other techniques in that specific entity of the mucin molecule is used as a

marker. Lectin and colorimetric methods are based on affinity for certain carbohydrates or for total glycoprotein. However, measurement of amino sugars does have at least one distinct advantages. The only source of interference with this technique would be compounds containing amino sugars whereas with, for example, the colorimetric methods, proteins and nucleic acids can also interfere (Mantle and Allen, 1989). Although many of the methods available for measuring mucin contents would not used, for obvious reasons considering the milieu of intestinal digesta, they have been mentioned here to demonstrate firstly some of the challenges faced in estimating the mucin content in ileal digesta, and secondly that, with exception of a complete purification or suitable immunoassays, this method is at least as efficient, if not more efficient, than other available methods.

B. Contribution of Mucin To Endogenous Protein

The contribution of mucin to endogenous protein was determined by estimating the mucin content of ileal digesta from pigs fed a protein-free diet. Overall, mucin represented 5 to 11% of endogenous protein, depending on the infusion treatment and the degree of proteolytic degradation However, the predominant amino acids in mucin, threonine, serine and proline, represents considerably higher proportions of their endogenous counterparts, 28 to 33%, 13 to 16% and 7 to 24%, respectively. These results are consistant with those obtained with protein-free diets, showing threonine to be the predominant indispensable amino acid (e.g., de Lange et al., 1989) in endogenous protein. The presence of mucin in ileal digesta will, therfore, explain the low digestibilities of this amino acid in many feedstuffs (Sauer and Ozimek, 1986). In addition, fermentation of mucin will account for the large disappearance of threonine in the large intestine. Despite the large proline content of mucin, this does appear to be the primary source of this amino acid in ileal digesta. The estimated recovery of endogenous proline based on the dilution of ^{15}N (Chapter VI) was closer to that

in ileal digesta of pigs recieving saline infusion, than in those infused with a complete amino acid mixture. In pigs infused with saline alone the contribution of proline in mucin to total proline in ileal digesta was only about 7%. The exact source of the proline in ileal digesta from pigs fed protein-free diets, without amino acid infusion, has not yet been determined, although the proline rich proteins from saliva may be one source.

Mucin was determined to be the primary source of endogenous carbohydrates in both the digesta from pigs fed a protein-free diet (Chapter II) and ileostomy effluent (Chapter III). A similar conclusion was drawn in several recent studies. Englyst and Cummings (1985, 1986, 1987) reported that fucose and galactose represented about 50% of the total neutral carbohydrates in ileostomy effluent from humans consuming NSP-free diets. Mucin carbohydrates, galactose, fucose and the amino sugars, account for 77% of the total carbohydrates at the distal ileum of colectomized rats fed fiber-free diets (Monsma et al., 1992).

C. Efect of Fiber on Mucin Output

The effect of the consumption of fibers on the daily output of mucin at the distal ileum was determined in two studies. The consumption of 1.1 to 33.7 g soy fiber/d in total enteral nutrition formulas did affected the daily output of mucin in ileal effluent. However, the design of the experiment created a unique continuum of soy fiber intake which, by regression analyses, demonstrated conclusively that nonmucin carbohydrates in crude mucin were derived from dietary NSP. The importance of this observation was expressed in the following experiment (Chapter IV) in which xylose and arabinose contributed more than 70% of carbohydrates in crude mucin. Extensive dialysis and gel-permeation chromatography on columns of Sepharose and Sephdex does not remove these contaminants (Clamp and Gough, 1991). The consumption of 0 to 240 g pea fiber/d resulted in a linear increase in the daily output of mucin in ileal digesta

of pigs. Daily mucin output increased from approximately 6 g/d with the basal wheat diet to approximately 7.5 g/d with the consumption of 240 g pea fiber/d, a 25% increase. These results, in conjunction with studies measuring the effect of the consumption of fiber on mucin secretion, support the proposition that physical abrasion is a primary reason for the presence of mucin in the intestinal lumen (Allen, 1981). Mucin secretion was higher in the stomach of rats adapted to diets containing 5% citrus fiber compared to those adapted to a fiber-free diet. Gastric mucin secretion was not elevated in rats adapted to 5% guar gum diets. Mucin secretion is higher in the intestine of rats adapted to diets containing fiber with insoluble fractions such as cellulose, wheat bran and citrus fiber (approximately 25% insoluble) (Vahouny et al., 1985; Satchithanandam et al., 1989, 1990) but not in rats adapted to diets containing completely soluble fibers such as guar gum and carrageenan (Satchithanandam et al., 1989, 1990). The effect of the abrasive action of dietary residuals on daily mucin output was further demonstrated by the observation that mucin output was influenced by dry matter output but not soy fiber intake despite the fact that the recovery of soy fiber in ileal effluent was essentially responsible for the increase in dry matter output (Chapter III). This indicates that mucin output responds to large variations in dry matter output.

Since the amount of mucin in the mucus layer represents a balance between seretion and erosion, a measure either does not provide an entirely accurate picture of the events that are occurring. To determine the state of the mucus layer a measure of both the secretion and degradation of mucus gels is required (Mantle and Allen, 1989). The importance of this, and the value of estimates of the mucin content in ileal digesta, are exressed in observations from other areas. Observations that mucin secretion is elevated by insoluble fibers but not soluble fibers (Vahouny et al., 1985; Satchithanandam et al., 1989, 1990) are in contrast to those of Cassidy et al. (1981) who observed that feeding diets supplemented with either 15% pectin or alfalpha to rats increased the percentage of intestinal villi exhibiting structural deviations by about 300% compared to rats

fed chow, while neither 15% wheat bran nor 15% cellulose had any effect. The difference in these results reflect the different responses of the intestine to these fibers.

The abrasive action of insoluble fibers appears to be countered by increasing the rate of mucin secretion (Satchithanandan et al. 1989, 1990). While neither 15% wheat bran nor cellulose appeared to have any effect on intestinal morphology, there was a visible increase in mucus and an apparent increase in goblet cell activity (Cassidy et al., 1981). The intestine responds to the consumption of soluble fibers such as guar gum and pectin by increasing crypt cell production rates (Jacobs, 1983; Johnson and Gee, 1986), presumably in response to damage such as that observed by Cassidy et al., (1981). Similar increases in crypt cell production were not observed for oat bran (Jacobs, 1983). Crypt cell production rates are increased in response to local cell damage (Rijke, 1976). Such damage would presumably require a substantial effect the mucus layer. The reaction of the intestine to soluble fibers, therefore, appears to be more dramatic, increasing the rate of cell replacement as opposed to the rate of mucin secretion. The mucosal barrier would be presumably be reestablished and maintained by mucin from newly differentiated goblet cells and the secretion of mucus from the crypt cells themselves. These differences are not reflected in measures of mucus secretion with soluble versus insoluble fibers (Satchithanandan et al., 1989, 1990), however they are implied by the relative daily outputs of mucin in ileal digesta. Monsma et al. (1992) observed a 34% increase in mucin carbohydrate output in colectomized rats following the consumption of a 5% gum arabic diet versus a fiber-free diet. This is a considerable increase considering the consumption of 15% pea fiber increased mucin output by only 25% (Chapter IV). The consumption of 15% guar might be expected to be induce a considerably higher output of mucin which would likely be achieved through considerable to the mucus layer and thus the underlying epithelium. These changes are not detected in observations with mucus secretion because rats are fasted for 24 h prior to measurements, allowing the intestine

sufficient time to repair itself (Silen and Ito, 1985). The effects may be somewhat different in the stomach, however, since guar gum, oat bran and pectin increase the proliferative zones in the crypts of the rat stomach (Lupton and Jacobs, 1987).

D. Estimation of the Recovery of Endogenous Protein With ^{15}N-Isotope Dilution Techniques

Despite the obvious advantages of estimating the recovery of endogenous protein in ileal digesta from different sources, a method for the determination of the total recovery of endogenous protein is required to fully assess the value of feedstuffs. Traditional methods for estimating the recovery of endogeous protein, feeding protein-free diets and the regression method, are unsuitable because they do not allow direct determinations for protein-containing diets. As such they lack the ability to determine changes in endogenous protein recovery as a result of dietary manipulation. As yet, the best method for determining the contribution of endogenous to total protein in ileal digesta of pigs fed protein-containing diets appears to involve the use of ^{15}N-isotope dilution techniques. However, resent results with the ^{15}N-isotope dilution technique have raised some concerns that must be addressed prior to further use of these techniques.

The first part of this study (Chapter V) sought to determine the effect of dilution in the dilution in the precursor pool for endogenous N secretion estimates of the recovery of endogenous protein in ileal digesta. While enrichments (atom % excess ^{15}N) in N were similar in all pools, enrichments in leucine, isoleucine, valine and alanine in the TCA-soluble fraction of plasma were at least twice that in the other pools. This was supported by recent observations of Souffrant et al. (1993). These authors reported enrichments in pancretic juice and urine which were twice that in either the TCA-soluble or insoluble fractions of plasma. These results demonstrated that the relative contribution of nonprecursor N, N that is not directly related to the secretion of endogenous N

in the intestinal lumen, was a major determinant of the observed enrichments in the TCA-soluble fraction of plasma. It was concluded, therefore, that the recovery of endogenous protein in ileal digesta was overestimated with the ^{15}N-isotope dilution technique, in its current form. It was also concluded that enrichments in the actual precursor pool for endogenous N secretion, predominantly the plasma free amino acids, urea and ammonia. need to be determined more directly.

As a consequence of this large fraction of nonprecursor N, the actual enrichments in N secreted into intestinal lumen are underestimated. As a result, the contribution of endogenous to total protein in ileal digesta was overestimated, 84% compared to 46% when determined by dilution in amino acids. These results explain the very high digestibilities determined with the ^{15}N-isotope dilution technique, especially those in wheat, barley and soybean meal (de Lange et al., 1990). Results presented in Chapter V clearly demonstrate fundamental difficulties with the ^{15}N-isotope dilution technique. These difficulties suggest that, in its current form (e.g. de Lange et al., 1990; Souffrant et al., 1993), this technique is not an adequate method for determining the recovery of endogenous protein in digesta collected from the distal ileum of pigs fed protein-containing diets. An accurate assessment of the recovery of endogenous protein, therefore, requires that measurements of the dilution of ^{15}N in protein needs to determined more directly.

In the second part of this study the recoveries of amino acids were determined more directly by dilution of ^{15}N in individual amino acids and these were compared to recoveries determined with the ^{15}N- and ^{15}N-leucine isotope dilution techniques, as described by de Lange et al. (1992). In addition, the effect of the pattern of blood sampling was also determined. Enrichments in crude mucin were compared to those in the TCA-soluble fraction of plasma to assess the validity of using this plasma pool to estimate enrichments in endogenous amino acids in ileal digesta. The results for this study are discussed in Chapter VI. The results from this study suggest that the TCA-soluble fraction of plasma is a valid

precursor pool for the synthesis of endogenous protein that is secreted into the intestinal lumen and that bacteria do not significantly modify enrichments in ileal digesta.

With repect to the methodology of the ^{15}N-isotope dilution technique, the most important observation was that enrichments in leucine and alanine in the TCA-soluble fraction of plasma, and thus estimates of their enrichments in endogenous protein in ileal digesta, are overestimated. The reason for this is two-fold. Firstly, blood samples taken only at the time of feeding do not take into account dilution of enrichments in plasma resulting from the absorption of unlabelled dietary amino acids following the meal. Since endogenous protein secretion is constant this dilution will be reflected in the enrichments in endogenous amino acids in ileal digesta. However, since the enrichments in most amino acids were unaffected by the pattern of blood sampling, this may be a relatively minor effect. More importantly, and with greater consequence to the methodology of the ^{15}N-isotope, is role of metabolic processes in determining enrichments in leucine at the time of feeding. Leucine enrichments are likely to be elevated in blood samples taken only the time of feeding since leucine transamination and oxidation rates are reduced in the postabsoptive state (e.g., Cotiella et al., 1988). In this respect, the relative rates of digestion, absorption, and metabolism of different feedstuffs will be important since they will determine the extent of the postabsorptive period, thereby influencing the retention of ^{15}N by leucine and thus its enrichment. Since the catabolism of leucine is controlled by plasma amino acid levels (Castellino et al., 1987; Motil et al., 1984), the rate of ^{15}N infusion (Tessari et al., 1985), food intake (Reeds and Fuller, 1983) and the quantity and quality of protein consumed (Harper and Benjamin, 1984; Fuller et al., 1987; Benevenga et. al., 1993) may also influence the enrichment in leucine and, consequently, other amino acids, at the time of feeding. Thus enrichments in free amino acids in plasma blood samples taken at feeding are, therefore, overly influenced by rates of digestion, absorption and metabolism of different feedstuffs, as opposed to simply providing a reliable estimate of the ^{15}N-

enrichment in endogenous protein. As a result, the sensitivity of isotope dilution techniques for estimating the recovery of endogenous amino acids in ileal digesta is compromised by taking blood samples only at feeding.

In light of these metabolic considerations, the pattern of blood sampling used in this study, although more representative of the enrichment in endogenous protein throughout the period digesta collection, may also provide an overestimate. The problem is that each hourly blood sample is given equal weight in assessing the enrichment in endogenous amino acids, however, endogenous protein synthesis and secretion might be expected to correspond with the presence of feed and dietary residuals in the digestive tract. Thus the importance of the enrichments in amino acids in the postabsorptive period may be overemphasized in blood samples taken hourly and pooled and will be influenced by the extent of the postabsorptive period. To minimize this effect diets should be offered more frequently, providing a more constant passage of digesta, synthesis and secretion of endogenous protein and absorption of amino acids.

An additional consideration is that not all amino acids, particularly the essential amino acids, exhibit significant incorporation of ^{15}N, therefore, endogenous recoveries for these amino acids are lacking. Given the cost of ^{15}N-labelled amino acids this may be a difficult problem to overcome. The best alternative may be the ^{15}N-leucine isotope dilution technique, as described by de Lange et al. (1992), since digestibilities with this technique were similar to that obtained by dilution in individual amino acids. However, further information regarding the composition endogenous protein is required. The advantage of this technique is assessment of the recovery of endogenous protein and amino acids would more feasable since a smaller quantity of [^{15}]leucine would be required.

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