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in Young Piglets and Preruminant Calves
Fed Milk Protein, Soybean Protein, or Soybean Protein
Plus Putrescine or Ethylamine

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Alan Leslie Grant

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SMALL INTESTINAL VARIABLES IN YOUNG PIGLETS AND PRERUMINANT CALVES FED MILK PROTEIN, SOYBEAN PROTEIN, OR SOYBEAN PROTEIN PLUS PUTRESCINE OR ETHYLAMINE

By

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ABSTRACT

SMALL INTESTINAL VARIABLES IN YOUNG PIGLETS AND PRERUMINANT CALVES FED MILK PROTEIN, SOYBEAN PROTEIN, OR SOYBEAN PROTEIN PLUS PUTRESCINE OR ETHYLAMINE

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Inclusion of soybean protein concentrate (SPC) in preruminant calf diets decreased intestinal xylose absorption, mucosal protein concentration, efficiency of protein synthesis, and crypt cell proliferation, but increased dipeptidase activity. Feeding putrescine or ethylamine with SPC reversed these changes. SPC and SPC plus putrescine reduced mucosal ornithine decarboxylase activity. Changes in lactase activity paralleled changes in protein and RNA:DNA.

Inclusion of soy protein isolate (SPI) in diets of piglets up to 14 days of age reduced xylose absorption and crypt depths and altered the distribution of polyamines in mucosa. Feeding putrescine or ethylamine corrected xylose absorption, but reduced protein, DNA, and RNA concentrations. Ornithine decarboxylase, dipeptidase, and sucrase were least in pigs fed putrescine. When additional isolate was fed to pigs up to only 7 days of age, DNA and RNA were less in pigs

fed SPI than in those fed putrescine, but other variables were not altered.

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LIST OF ABBREVIATIONS

AMP: all-milk protein milk replacer

d: day(s)

DNA: deoxyribonucleic acid

g: gram(s)

h: hour(s)

kg: kilogram(s)

mg: milligram(s)

min: minute(s)

ml: milliliter(s)

nmole: nanomole(s)

ODC: ornithine decarboxylase

pmole: picomole(s)

Put: putrescine

RNA: ribonucleic acid

sec: second(s)

SEM: standard error of the mean

SPC: milk replacer with soybean protein concentrate

Spd: spermidine

SPE: milk replacer with soybean protein plus ethylamine

SPI: milk replacer with soybean protein isolate

Spm: spermine

SPP: milk replacer with soybean protein plus putrescine

um: micrometer(s)

INTRODUCTION

The polyamines, putrescine, spermidine, and spermine are necessary for small intestinal mucosal growth and development (Luk et al., 1982). When putrescine or the amine, ethylamine is infused into the rat small intestine or when the amine, dimethylamine, is fed to rats, small intestinal mucosal growth is stimulated (Dembinski et al., 1984; Seidel et al., 1985). Conversely, inhibitors to polyamine biosynthesis reduce small intestinal mucosal growth and development, e.g. crypt cell proliferation, villus growth, and development of mucosal digestive enzymes (Luk et al., 1982).

Abnormal mucosal growth and reduced digestion and absorption of nutrients is often reported when calves or piglets are fed milk replacers containing soybean protein (Seegraber and Morrill, 1979, 1982, and 1986; Silva et al, 1986a and 1986b). These effects may be due to the presence of soybean trypsin inhibitors, allergens, lectins, or other inadequately identified factors. The idea that soybean proteins contain inhibitors to polyamine biosynthesis is speculative and warrants investigation. If soybean proteins do contain inhibitors to polyamine biosynthesis, then supplementing milk replacers containing soybean protein with amines may reverse the resulting abnormalities in small

intestinal function. Even if soybean proteins do not inhibit polyamine biosynthesis, amines may stimulate mucosal growth and development so as to make milk replacers containing soybean proteins satisfactory for young pigs and preruminant calves. These ideas are addressed in the objectives to follow.

The first objective of experiments 1, 2, and 3 was to compare the effects of all-milk protein milk replacers and milk replacers with 20% or 50% of the protein replaced with various processed soybean proteins on young piglet or preruminant calf performance and on the following small intestinal variables: absorption, mucosal morphology, cytology, digestive enzyme concentration, and polyamine biosynthesis.

The second objective was to examine the effects of ethylamine or putrescine, when added to milk replacers containing these soybean proteins, on these intestinal variables.

LITERATURE REVIEW

I. DIGESTION AND ABSORPTION IN THE YOUNG PIG AND PRERUMINANT CALF

A. PROTEIN DIGESTION AND ABSORPTION

1.Gastric digestion of protein:

Protein hydrolysis in young pigs and preruminant calves require digestive enzymes of the abomasum, the pancreas, and the small intestine. Major proteolytic activity in the abomasum is due to actions of hydrochloric acid, pepsins, and chymosins. Secretion of hydrochloric acid by parietal cells is very low in neonates (Toullec et al.,1983), thus pepsins and chymosins become the major proteases in the neonatal abomasum. However, the optimum pH for hydrolysis is 2.0 for pepsin and 3.6 for chymosin (Jenkins et al., 1980). The pH of abomasal contents soon after feeding is about 6.1 and does not decrease to 2 to 3.6 until several hours after feeding indicating that proteins which do not form chymosin clots (plant and fish proteins) and leave the abomasum before the pH decreases are not hydrolyzed as extensively as proteins such as casein (Jenkins et al., 1980). Whereas both the pepsins and the chymosins have coagulating and proteolytic activities, the chymosins have several fold more coagulating activity and much less proteolytic activity than the pepsins

(Toullec et al., 1983). At birth, the amount of chymosin in the abomasal mucosa is very much greater than that of pepsin. The amount of chymosin per gram of abomasal mucosa decreases after birth whereas that of pepsin remains the same until about 10 weeks after birth and then increases. The total amount of chymosin does not change but that of pepsin always increases with age. On a liveweight basis, the amount of chymosin is maximal at 1.5 days of age and decreases thereafter while pepsin increases to 21 days and does not change thereafter (Guilloteau et al., 1984).

In young pigs, proteinase activity of stomach tissue (units per gram of tissue) changes little for the first two weeks after birth and then increases rapidly to 8 weeks of age (Hartman, et al., 1961). Lindemann et al.(1986) found that the increase in the total gastric mucosa protease activity between weeks 5 and 6 of age was due to an increase in both tissue weight and enzyme activity per gram of tissue.

2. Small intestinal digestion and absorption of protein:

In the small intestine, the final products of protein digestion are oligopeptides and amino acids and result from actions of enzymes of the pancreas and the intestinal epithelium. Pancreatic proteases include endopeptidases (such as trypsin, chymotrypsin, nuclease, and elastase) and exopeptidases (carboxypeptidases). Of these, trypsin and chymotrypsin have received the most attention in piglets and preruminant calves. The pancreas synthesizes trypsinogen and chymotrypsinogen which are secreted into the duodenum via the

pancreatic duct. These are biologically inactive zymogens until they are converted into the active forms (trypsin and chymotrypsin) in the small intestine by enterokinase, an enzyme produced by the brush border of the small intestine (Alpers, 1986; Magee and Dalley, 1986). The function of these pancreatic proteases is to hydrolyze proteins to oligopeptides. They have only trace amounts of aminopeptidase activity and therfore have little to do with hydrolyzing the oligopeptides to amino acids (Adibi and Kim, 1981).

Chymotrypsin and trypsin activities (specific and total activities and total activity per kg liveweight) in the pancreas both increase rapidly after 1.5 days of age in the calf. Activities also increase with age in the duodenal contents (Brown et al., 1981; Gorrill et al., 1968) and the pancreatic juice (Ternouth et al., 1976) of preruminant calves and lambs. McCormick and Stuart (1967) and Ternouth et al.(1967) also noted that the volume of pancreatic fluids secreted by calves increased markedly with age.

Hartman et al.(1961), and Owsley et al. (1986) observed similiar trends in young pigs. Proteinase activities of the pancreas and the small intestinal contents increased with age up to 8 weeks. Corring et al. (1978) noted that total trypsin activity remains low for the first 4 weeks of age while chymotrypsin begins to increase during the first week of life in the pig pancreas. Specific activities of chymotrypsin and trypsin decreased from birth to 2 weeks

(chymotrypsin) and to 3 weeks (trypsin) and then increased up to 6 weeks for chymotrypsin and 8 weeks for trypsin.

In weaned ruminants, in which large quantities of ruminal microorganisms enter the intestine, nuclease is more abundant in the pancreatic juice than it is in nonruminants (Magee and Dalley, 1986).

The intestinal epithelium produces a variety of peptidases which are located in both the brush border of the small intestine and the cytosol of the intestinal epithelial cells (Adibi and Kim, 1981; Alpers, 1986). The function of these peptidases is to hydrolyze the oligopeptides, which have been formed by the action of the abomasal and pancreatic proteases, to shorter oligopeptides or amino acids. Distribution of peptidase activity in the ruminant small intestine has been studied recently in sheep (Richardson and Jouan, 1986), but none or very few studies have been conducted in calves. Richardson and Jouan (1986) found that dipeptidase activity was mainly associated with cytoplasm of the enterocyte, tripeptidase activity was distributed evenly distributed between the brush border and cytosol, and tetrapeptidase and longer-chain peptidases with the brush border. Activity was lowest in the proximal duodenum, increased through the jejunum, peaked in the mid-ileum, and declined towards the terminal ileum. Adibi and Kim (1981) have reviewed data showing that amino acid accumulation rate is greater in the lumen of the ileum than the jejunum after perfusion of an amino acid test solution due to two reasons : 1) slower rate of absorption of amino acids in the ileum and

2)greater peptide hydrolase activity in the ileum causing more rapid hydrolysis of peptides and greater back diffusion of amino acids into the lumen. Richardson and Jounn's data (1986) is consistent with that of Ben-Ghedalia et al. (1974) in that the maximum peptidase activity present in the first two-thirds of the small intestine corresponds to the region of maximum net disappearance of peptide nitrogen. Symons and Jones (1966) examined the distribution of peptidase activity in ovine small intestine and concluded that dipeptidase activity increases from proximal duodenum to mid-ileum and then declines towards the terminal ileum. In adult pigs dipeptidase activity is low in the proximal duodenum, gradually increases to reach a maximum in the jejunum and proximal ileum, and then decreases slightly throughout the remaining ileum (Josefsson and Lindberg, 1965).

The amount of intact peptide escaping hydrolysis by the brush border and cytosol in the intestine and reaching the blood is not clear. There are distinct transport systems for the absorption of peptides and amino acids (Magee and Dalley, 1986). In the newborn, proteins are obviously absorbed (before being hydrolyzed) in the form of immunoglobulins from ingested colostrum. However, closure of the intestine to the passage of the colostral immunoglobulins occurs between 36 and 48 hours after birth (Matte et al., 1982). Alpers (1986) states that approximately 25% of the total amino acids absorbed by the blood of mammals is in the form of dipeptides and tripeptides. Webb (1986) has shown in weaned calves that

more than 70% of the portal plasma amino acids are associated with the peptide fraction. However, source of these peptides has not been established.

B. CARBOHYDRATE DIGESTION AND ABSORPTION

1. Small intestinal digestion and absorption of carbohydrates:

Carbohydrate digestion and absorption in young pigs and preruminant calves require pancreatic and small intestinal carbohydrases. Pancreatic alpha-amylase is secreted into the intestinal lumen and hydrolyzes starch to maltotriose, maltose, and alpha-limit dextrins. These products plus the disaccharides, lactose and sucrose, are further hydrolyzed by enzymes of the intestinal brush border membranes. Gray (1981) discussed the proposed association of carbohydrates and monosaccharide carriers with the brush border surface membrane. He stated that the active sites of the enzymes are exterior to the brush border lipid bilayer, where they are readily available to substrates, and are anchored into the brush border by a hydrophobic segment, whereas monosaccharide transporters are located within the brush border membrane. Gray and Santiago (1966) showed by human comparative perfusion studies of dissacharides that surface hydrolysis of maltose and sucrose is very efficient suggesting that surface digestion is not rate-limiting and that the transport process probably is rate-limiting in the assimilation of polysaccharides. However, lactose was found to be hydrolyzed

so inefficiently that uptake of galactose and glucose was not maximal suggesting that hydrolysis appeared to be rate-limiting in the assimilation of lactose in normal adult humans. Furthermore, in many adult African and American Blacks, Orientals, and Mexicans lactase is even further reduced resulting in even greater maldigestion at the intestinal surface. In neonates, lactase activity is greater, thus lactose hydrolysis may not be as limiting in the assimilation of lactose as it is in the adult (discussed later in this review).

The major difference between young pigs and calves in the assimilation of carbohydrates is the absence of sucrase in the ruminant species (Dollar and Porter, 1957). The preruminant calf therefore does not have the ability to utilize sucrose as a carbohydrate source unless fermentation of sucrose by microorganisms in the lower digestive tract allows the sucrose to be utilized (Morrill et al.,1965). Sucrase activity in the piglet is almost nonexistent in the newborn, but increases greatly with age until maturity (Manners and Stevens 1972). Activity varied along the intestinal tract and maximal activity (per gram of mucosa) was found at sites that were at 20% and 40% of the length of the small intestine in pigs from 1 to 8 weeks of age. For pigs over 5 weeks of age, maximum activity was at sites 40% and 60% along the tract.

Lactase activity per gram of mucosa in the calf and pig is high at birth and then declines with age (Dollar and

Porter, 1957). Huber et al (1961) reported that the 6 week old calf had 6-fold less lactase activity than the newborn. Manners and Stevens (1972) reported that lactase activity in pigs decreased from birth to 8 weeks of age and did not change thereafter. However, others (Jones, 1984) reported that activity remained high for the first two to three weeks of age. Maximum activity was found in the proximal one-third of the intestine peaking at the site 20% along the length (Aumaitre and Corring, 1978; Ekstrom et al., 1975; Manners and Stevens, 1972).

Total and specific pancreatic amylase activity in the calf is very low at birth and decreases to 1.5 days of age, but increases rapidly thereafter for several weeks depending on when the calf is weaned (Guilloteau et al., 1984; Huber et al. 1961). Total activity in the pig increases rapidly with age from birth to weaning (Owsley, 1986) due to increases in pancreas weight and specific activity of the enzyme (Corring et al ., 1978; Lindemann et al ., 1986).

Intestinal maltase activity follows the same trends and like amylase tends to increase markedly with age, but maltase activity in ruminants is less than in swine (Aumaitre and Corring, 1978; Catron et al., 1957; Huber et al., 1961) especially at the time of weaning when the consumption of starch is increased (Huber et al., 1961).

C. THE EFFECT OF DIET ON DIGESTION AND ABSORPTION OF PROTEINS AND CARBOHYDRATES IN THE YOUNG PIG AND PRERUMINANT CALF

1. The use of soybean protein in milk substitute diets:

The poor performance exhibited by young pigs and preruminant calves receiving soybean protein as a substitute for milk protein has been attributed to (1) the rapid increased passage of digesta through the gastrointestinal tract (Guilloteau et al., 1981; Sissons and Smith, 1976; Ternouth et al., 1974; Ternouth et al., 1975), (2) reduced extents of dry matter and crude protein digestibilities and reduced absorption (Campos et al., 1982; Campos and Huber, 1983; Huber and Campos, 1982; Huber et al., 1984; Lister and Emmons, 1976; Schingoethe and Thomas, 1969; Silva et al., 1986a; Sissons and Smith, 1976;) and (3) intestinal allergies to soybean proteins (Kilshaw and Sissons, 1979a, Kilshaw and Sissons, 1979b Kilshaw and Slade, 1980,).

Garnot et al. (1972 and 1977) and Williams et al. (1976) found that chymosin concentrations in the mucosa and digesta of the abomasum were reduced when preruminant calves were fed soybean protein rather than milk protein. This would lead one to expect that curd formation would be reduced and passage out of the abomasum increased. Emmons and Lister (1976) in studying factors affecting in vitro curd formation found that curd firmness was increased by higher chymosin concentrations. Emmons et al. (1976) and Ternouth et al. (1975) found that when soybean meal, soy protein isolate, and soybean flour were added to reconstituted skim milk powder curd firmness was markedly reduced. They also observed more rapid passage of ingested protein out of the abomasum for a diet containing soybean flour as compared to one containing

skim milk and whey protein. There was no difference in the rate of abomasal outflow of the whey fluids between diets. Jenkins et al. (1980) stated that plant proteins do not form a chymosin clot and are expelled from the abomasum before the pH decreases sufficiently for effective pepsin activity. Guilloteau et al. (1981) infused milk replacers slowly or quickly into the duodenum to simulate gastric emptying rates of diets with different protein sources and observed a reduced apparent digestibility of dry matter, lipid, minerals, and protein and greater blood free amino acid levels when diets were infused quickly. These results suggest that digestion of nutrients and metabolic utilization of amino acids is highly infuenced by the time spent in the abomasum and the rate of arrival in the duodenum.

When preruminant calves were fed milk replacers with two-thirds of the milk protein replaced with hot ethanol treated soybean protein or heated soybean flour weight gains and feed efficiencies were reduced (Silva et al., 1986a and 1986b) These reductions were attributed to reduced organic matter and crude protein digestibilities and to reduced absorption of nutrients. Calves receiving soybean protein absorbed 16% less xylose than those receiving all milk protein diets after they were challenged with an oral dose of xylose. Campos et al. (1982) found that organic matter digestion and nitrogen retention was reduced in calves when soy protein concentrate replaced milk protein. Similiar results with xylose absorption tests occurred in calves with

one-third of their dietary protein replaced with soy protein concentrate or soy flour (Seegraber and Morrill, 1982). In addition, the consumption of soybean protein and the reduced xylose absorption was associated with the absence of or blunted, convoluted villi (Seegraber and Morrill, 1982 and 1986).

Nitsan et al. (1971) suggested that the inferior growth performance of calves fed soybean protein was due to reduced utilization of absorbed nitrogen rather than reduced protein digestiblity. They found no difference in amount of absorbed nitrogen between calves given raw or partly heated soybean replacers and calves given heated or all-milk replacers. However, calves given raw or partly heated soybean had higher blood urea nitrogen and lower blood glucose suggesting that raw or partly heated soybean results in a disturbed assimilation of absorbed nitrogen possibly due to a shortage of available carbohydrates essential for optimal nitrogen utilization.

Sissons and Smith (1976) examined the effects of cows milk, a casein protein milk replacer, and various soybean protein milk replacers on digesta movement and water and nitrogen absorption in the small intestine of the pre-ruminant calf. Passage of digesta and absorption of nitrogen and water did not differ between the milk and the casein diets. However, a diet based on heated soybean flour fed for the first and second time resulted in greater ileal flow rates and lower net nitrogen absorption. Unheated soybean flour resulted in even a lower nitrogen absorption. After

feeding soybean flours for several feedings passage of fluid in calves was reduced indicating inhibition of abomasal emptying. Severe digestive disturbances were observed suggesting that these results were due to gastrointestinal allergies. Diets of water-extracted soybean flour and of soybean protein isolate, but not of soybean protein concentrate (ethanol extract), showed similiar results. Since heat treatment of soybean flour removes most of the trypsin inhibitors and haemagglutinin activity (Wolf, 1967), the negative results obtained from heated soybean flour must not be due to trypsin inhibitor or haemagglutinin. Extraction of soybean protein with ethanol results in a protein which does not cause digestive disturbances which suggest that ethanol extracts or destroys a detrimental factor (Sissons and Smith, 1976).

Pancreatic fluid secretion and chymotrypsin, trypsin, and amylase activities of the fluid were lower in preruminant calves fed diets containing soybean flour relative to those fed skim milk and whey powder (Gorrill et al., 1967). Soybean protein fed to such calves failed to yield the pancreatic hypertrophy and hypersecretion of digestive enzymes (Gorrill and Thomas, 1967) that have been observed in rats and chicks (Garlich, 1966; Rackis, 1965). Efird et al. (1982) noted that piglets fed soybean protein had longer intestines, faster growing pancreases, greater trypsin and chymotrypsin activities in the intestinal contents, and lower activities in the pancreas compared to milk-fed pigs

suggesting that soybean protein causes greater secretion of trypsin and chymotrypsin into the intestine. Reduced animal performance from increased pancreatic enzyme secretion has been explained two ways. Gertler et al. (1967) suggested that the increased secretion which is accompanied by increased pancreatic enzyme synthesis leads to a loss of endogenous nitrogen and thus increases the amino acid requirement of the animal. The reduction in protein utilization as a result of feeding the soybean protein further antagonizes the shortage of amino acids. Alpers and Tedesco (1975) demonstrated in vivo that degradation of brush border enzymes is positively associated with an increased concentration of luminal pancreatic proteases. Goda et al. (1985) have shown that sucrase activity in the rat jejunum is influenced by trypsin. Thornburg et al. (1987) suggested that dietary-induced changes in rat intestinal disaccharidase activities may be due to changes in proteolytic activity of pancreatic secretions. However, Hooks et al. (1965) and Pekas (1966) reported that the young pig responds to soybean protein similiar to calves in that pancreatic hypertrophy and hypersecretion of juice does not occur. Ternouth et al. (1975) observed no reduction in pancreatic fluid flow and pancreatic enzyme activities when soybean flour was substituted for part of the skim milk powder in calf diets, however, the soybean flour had been treated to inactivate the trypsin inhibitor. Gorrill and Thomas (1967) compared two soybean protein diets, one containing large amounts of trypsin inhibitor and the other containing neglible amounts,

and concluded that the reductions in body weight, pancreatic enzyme activities, and in vitro digestion of intestinal protein from calves fed soybean protein with large amounts of soybean trypsin inhibitor were due only in part to the presence of trypsin inhibitor. Subsequently, Schingothe et al. (1970) were able to seperate a factor, possibly a glycoprotein, from raw soybean meal which was devoid of trypsin inhibitor, but decreased weight gains in mice without affecting the size of the pancreas.

Methionine has been reported to be the most limiting amino acid in soybean protein (Hays et al., 1959). Attempts to improve animal performance by supplementing soybean diets with methionine have shown some success. Gorrill and Nicholson (1969) were unable to improve growth and nitrogen retention of calves when milk replacers containing 70% of the protein from soy protein concentrate were supplemented with DL-methionine (0.1% of diet dry matter or 0.5g/100g diet protein). Conversely, Porter and Hill (1964) were able to improve growth in calves receiving methionine-supplemented isolated soybean protein, but they supplemented the diets with higher amounts of methionine (0.8g/100g diet protein). Hays et al. (1959) increased weight gains and feed efficiencies in 10 day old pigs by supplementing soybean diets with DL-methionine (0.05% of dry matter or 0.25g/100g diet protein). They found that the improved performance was due to higher dry matter and protein digestibilities.

Walker et al. (1986) studied effects of soybean protein

as a substitute for milk protein on performance, digestibility, and amino acid availability in 21-day weaned pigs between 3 and 8 weeks of age. They found that digestibilities of dry matter and various amino acids and the availability of nitrogen and amino acids were greater for pigs consuming casein diets than for those receiving soybean protein diets. They concluded that these differences accounted for higher average daily gains and feed to gain efficiencies in casein-fed pigs. Several other studies have shown that partial substitution of milk protein by soybean meal, soy flour, or partially hydrolyzed soy flour products reduced weight gains and feed effficiencies in preweaned pigs younger than 21 days of age (Jones et al., 1977 ;Wilson and Leibholz,1981;Zomora and Veum, 1978). Pekas et al. (1964) found that protein and dry matter digestibility reductions by exclusion of panreatic secretion from the duodenum were greater for neonatal piglets fed solvent extracted soybean meal than for those fed dried skim milk as a source of protein. They also found that the efficiency of the whole digestive system, excluding the changes in pancreatic fluid improved considerably with advancing age when soybean protein was fed, but had a lesser degree of improvement when fed milk protein. A similiar digestive compensation was reported in pigs by Corring and Bourdon (1977).

2. The association of soybean protein with gastrointestinal allergies:

Gastrointestinal allergies to soybean protein have been

shown to exist in young calves (Kilshaw and Sissons, 1979a and 1979b; Kilshaw and Slade, 1980 and 1982). Preruminant calves receiving a liquid diet containing heated soybean flour developed high titres of serum antibodies specific for soybean proteins within two weeks (Kilshaw and Sissons, 1979a). Production of these antibodies coincided with the increase in the rate of flow of digesta through the gastrointestinal tract. Kilshaw and Sissons (1979b) observed that when calves were fed soybean flour diets high titres of serum antibodies were produced with specificity for the soybean proteins, glycinin and beta-conglycinin. When soybean flour diets containing these two proteins were fed to calves development of digestive disturbances and production of serum antibodies occurred. Kilshaw and Slade (1982) noted that calves receiving a single feeding of a diet containing heated soybean flour caused intestinal villus atrophy and crypt elongation within 24 hours and that successive feeding of the diet caused increased villus atrophy, crypt elongation, and severe diarrhea. Silva et al., (1986b) observed that soy protein concentrate caused greater villus atrophy and poorer health in calves which had previously been exposed (sensitized) to the soybean protein. Barratt et al.(1978) also noted in calves and pigs that heated, ethanol-extracted soybean meal resulted in villus atrophy and lymphocytic infiltration. Disodium cromoglycate (a drug used to prevent immediate hypersensitivity reactions) and indomethacin (a drug used to alleviate symptoms of food

intolerance) were unable to prevent digestive disturbances in calves receiving soybean flour arguing that gastrointestinal allergies result indirectly from soybean proteins and are a consequence of gastrointestinal malfunction, i.e. altered digestion and/or inadequate synthesis of secretory immunoglogulins IgA and IgM and subsequent increased mucosa permeability to antigens (Barratt and Porter, 1979; Kilshaw and Sissons, 1979a; Kilshaw and Slade, 1980).

II. DEVELOPMENT OF SMALL INTESTINAL EPITHELIUM

A. MUCOSAL ARCHITECTURE AND EPITHELIAL CELL KINETICS

1. Variability between species:

In the small intestine, epithelial cells of the tips of villi are continuously sloughed off and replaced by continuous migration of crypt cells onto and along villi. Crypt cells are actively proliferative and differentiate into villus cells. These include digestive, absorptive, Paneth (Paneth cells are absent in cats, dogs, and pigs; Magee and Dalley, 1986), endocrine, and goblet cells (Trier and Madara, 1981). The time required for epithelial cells to complete this cycle, i.e. from DNA synthesis in the crypts to arrival at the villi tips, is referred to as the replacement time, whereas the average distance migrated per unit time is referred to as the migration rate (Moon and Skartvedt, 1975). Postnatal changes in these two parameters, as well as postnatal changes that occur in the number and the size of villi and crypts, vary among species.

Crypt depth increases during the first few weeks after

birth in rats (Koldovsky et al., 1966), pigs (Moon, 1971), chickens (Moon and Skartvedt, 1975), and in calves and lambs (Moon and Joel, 1975). Number of crypts has been shown to increase postnatally in rats (Clarke, 1972) and in chickens (Clarke, 1967) which indicates that the size of the proliferating compartment for intestinal epithelial cells enlarges with advancing age. Duodenal crypts were longer than jejunal or ileal crypts in chickens (Moon and Skartvedt, 1975). The length of villi in chickens and rats increase with animal age whereas the number of villi remain the same (Clarke, 1972 and 1967; Koldovsky et al., 1966; Moon and Skartvedt, 1975). Even though the population to be replaced is increasing, the increase in cell production rate and migration rate leads to a reduction in the replacement time with advancing age in the rat (Koldovsky et al., 1966). However, in chickens villi elongation changes more rapidly than cell production rate resulting in an increased replacement time (Moon and Skartvedt, 1975). Moon and Skartvedt (1975) also observed that duodenal villi were longer than jejunal villi and jejunal longer than ileal villi. Because migration rate in the duodenum was greater than in the jejunum which was in turn greater than in the ileum, replacement time was actually lowest in the duodenum followed by the jejunum and then the ileum. In pigs, villi and crypts increase in number, but the deeper crypts and the shorter villi are associated with a marked decreased replacement time during the first few weeks of age (Moon,

1971). Similiar results were noted by Moon and Joel (1975) in calves and sheep in that villi length decreased, crypt depth and number increased, and replacement time decreased somewhat with age. Villi of the jejunum were also found to be shorter than those of the duodenum and ileum and migration rate tended to be greater in the more proximal sites of the small intestine in calves and sheep.

B. FUNCTIONAL CHANGES ASSOCIATED WITH THE DEVELOPMENT OF ENTEROCYTES

1. Loss of antibody transfer:

El-Nageh (1967) proposed that in the calf, closure, or cessation of antibody transfer, occurred as a result of intestinal epithelium being replaced with new cells incapable of absorbing immunoglobulin. Moon and Joel (1975) reported that less than half of the villus epithelial cells in the small intestine were replaced during the first 48 to 72 hours of age, indicating that closure, which occurs 24 to 36 hours of age, occurs before complete replacement of the villous epithelium. This is inconsistent with El-Nageh's hypothesis (1967) unless absorption of colostral protein is confined to the cells at the tips of villi which are replaced first as suggested by Moon and Skartvedt (1975). Smeaton and Simpson-Morgan (1985) determined, by serial biopsies of the small intestine from a single lamb 0 to 72 hours after 3H-thymidine labelling, that the epithelium was renewed within 2 to 3 days of age in the lamb. This is consistent with El-Nageh's hypothesis (1967) and may be inconsistent with results

reported by Moon and Joel (1975) due to animal variation in their data. These authors used a different animal for each hour sample and thus could have variability due to animal differences. Smeaton and Simpson-Morgan (1985) observed that immediately after birth intestinal epithelium of the lamb began to be replaced by a digestive type of cell, displacing cells capable of absorbing intact colostral protein.

In the newborn pig, Moon (1971) reported that 7 to 10 days were required for small intestinal villus epithelium to be replaced. Since closure occurs 24 to 36 hours after birth, closure must occur before complete replacement of epithelial cells in pigs.

2. Development of digestive and absorptive functions:

Undifferentiated crypt cells are the most abundant type of cell in crypts of the small intestine. They are columnar in shape with basally located nuclei. During mitosis chromatin migrates toward the luminal half of the cells. Daughter cells resulting from the mitotic activity of crypt cells migrate up the wall of the crypt and associated villi, cells become taller, and then begin to differentiate into absorptive cells. By the time the cells have reached the villus-crypt junction, they begin to resemble absorptive cells,i.e. the microvillus membrane becomes larger and is associated with the sequential increased amount of enzymes and transport proteins (King et al., 1983; Pearse and Riecken, 1967; Trier and Madara, 1981). Dahlqvist (1967) and Yamada et al.(1981) found that disaccharidase activities in

rat small intestinal mucosa were greatest in the distal parts of villi indicating that enzymes are being developed while cells migrate up the sides of the villi. This is consistent with the distribution of transporting activity which is confined primarily to the villus tip (Cheeseman et al., 1983).

Cheeseman (1986) examined the distribution of amino acid and peptide transport along the villus of rat small intestine and found that age of enterocytes is a determinant of their transporting ability. Expression of transport was always initiated in cells 30 to 35 hours of age, regardless of the cells' migration rate and position on the villus.

C. FACTORS AFFECTING ENTEROCYTE DEVELOPMENT AND FUNCTION

1. Effects of diet:

Feeding soybean protein to young calves has resulted in reduced absorption of xylose in several experiments (Seegraber and Morrill, 1979, 1982, and 1986; Silva et al., 1986b). Reduction in absoptive ability has been attributed to changes in intestinal morphology in some studies. Kilshaw and Slade (1982) and Seegraber and Morrill (1982 and 1986) observed villus atrophy and crypt elongation in the small intestine of calves which received modified soybean protein (heated ethanol-extracted) and heated soybean flour as part of their milk replacer compared to calves receiving all milk protein replacers. Seegraber and Morrill (1982 and 1986) observed a reduction in xylose absorption in these soybean protein fed calves and concluded that this was due to villus

atrophy. However, contradictory results were shown by Silva et al. (1986b). They fed modified soybean protein and heated soybean flour to calves and found a reduction in xylose absorption, but observed greater morphological variation in size and shape of villi within animals than among treatments. Crypts were not examined in the mucosa of these calves, so it is not known whether or not crypt elongation was occurring as was shown by Kilshaw and Slade (1982).

Menge et al. (1981) reviewed data from studies in rats with crypt hyperplasia and suggested that reduction in uptake of substrates by the enterocytes compared to that by normal enterocytes was due to less transport sites as a result of a less mature cell population. They present evidence demonstrating that increased proliferation is accompanied by a reduction in life-span of epithelial cells, thus leading to a shorter transit time of enterocytes through the maturation compartment resulting in lower levels of brush border enzyme activity. Menge et al.(1981) assumes that transport activity parallels brush border enzyme activity. Results of Cheeseman (1986) support Menge's hypothesis in that he found that enterocytes less than 32 hours old were unable to initiate transport of amino acids and peptides.

Adaptive changes in intestinal enzyme activities in response to changes in intake of carbohydrate have been shown mostly in rats, but also in ruminants. Huber et al. (1964) slowed down the postnatal decline in lactase activity in intestinal mucosa of calves by feeding a high-lactose ration to 11 weeks of age. High-lactose fed calves had 3 times

higher lactase activity than did control calves. Goda et al. (1983) were able to decrease lactase, sucrase, maltase, and glucoamylase activities in the microvillus of the rat jejunoileum by reducing the starch content of isocaloric diets. Yamada et al. (1981) also showed that rat jejunal sucrase and lactase activity was inceased with increased dietary starch. Furthermore, changes in activities in experiments by Goda et al. (1983) were observed within 24 hours in both mature and immature enterocytes indicating that enterocytes that leave the villus-crypt junction are not committed to achieve a predetermined amount of disaccharidase activity at various positions up the villus and that the change of activity is not dependent on production of new enterocytes from crypts. Sucrose, lactose, glucose, fructose, and galactose elicited similiar results in rats (Koldovsky et al., 1982). However, Yeh et al. (1986) concluded that the increase in rat intestinal sucrase activity observed after feeding sucrose diets was due to endogenous cortisone rather than dietary sucrose. Brush border and cytosolic peptidase activities (leucylnaphthylamidase and L-phenylalanylglycine hydrolase) were not changed in response to a change in starch intake except for a slight temporary decrease in the proximal jejunum. These changes may have been due to an increased migration rate of enterocytes, which was observed in the proximal jejunum at that time, which subsequently may have decreased the number of mature enterocytes thus decreasing

the amount of peptidase activity (Goda et al.,1983).

2. Effects of hormones, growth factors, and intestinal polypeptides:

Johnson (1982) discussed two types of stimulation for gastrointestinal growth and concluded that one is the involvement of non-gastrointestinal hormones and the other is the involvement of factors that result from the ingestion of food.

Examples of non-gastrointestinal hormones involved in the growth and development of enterocytes are the glucocorticoids and insulin. Many studies have shown that intravenous administration of glucocorticoids to rats increases activities of sucrase, maltase, and aminopeptidase and decreases lactase activity while adrenolectomyzing delays the usual increase in sucrase, maltase, and aminopeptidase and the decrease in the lactase during the first two weeks of age (Henning, 1985 and Yeh et al., 1986). Furthermore, changes in enzyme activities due to glucocorticoids were mediated via the crypt cells; the cells that are on the villi at the time of administration are unaffected by the hormone. The rate at which the altered enzyme activity appears in the villi correlates with the epithelial cell migration rates. In vitro, insulin added to isolated brush borders from one-week old rats had no effect on brush border enzyme activity. However, insulin increased the dexamethasone-stimulated activity of brush border enzymes suggesting a permissive role of insulin in the development of the digestive function of

intestinal enterocytes (Kedinger et al., 1982).

Examples of other factors involved in the growth and development of intestinal epithelium are gastrointestinal hormones and polypeptides (such as enteroglucagon and cholecystokinin) and other factors (such as epidermal growth factor). Al-Mukhtar et al. (1982) in studying intestinal adaptation in rats found that luminal nutrition(luminal exposure to different nutrients) in intact small intestine changed crypt cell production rate in Thiry-Vella intestinal loops (that were not exposed to the nutrients) indicating that there could be another factor(s) which is important in intestinal adaptation. They found a significant correlation (r square = 0.65, $p < .02$) between plasma enteroglucagon and crypt cell production rate in the ileum. There was also positive correlation between plasma enteroglucagon concentration and presence of food in the ileum supporting the hypothesis that presence of food in the ileum stimulates enteroglucagon release which subsequently stimulates intestinal proliferation.

Lorenze-Meyer et al. (1982) infused cholecystokinin (CCK) intravenously into growing rats and observed increased concentrations of DNA and RNA, increased specific activity of sucrase, decreased lactase activity, and increased villus surface area in jejunal mucosa. Injection of CCK also increased L-phenylalanine uptake indicating that CCK affects the growth of intestinal mucosa as well as the development of enterocytes.

Dembinski et al. (1982) demonstrated that epidermal

growth factor (EGF) given either parenterally or luminally to mature rats significantly increased duodenal mucosa DNA synthesis and DNA concentration. Malo and Menard (1982) administered EGF to suckling mice and observed a slight increase in brush border hydrolase activity. However, Kedinger et al. (1982) did not observe any changes in rat intestinal brush border enzyme activity after administration of EGF to an in vitro system.

III. POLYAMINES

A. METABOLISM OF POLYAMINES

The polyamines, putrescine, spermidine, and spermine are ubiquitous (i.e. they are found in and are synthesized by all nucleated prokaryotic and eukaryotic cell types) and are organic polycationic compounds of low molecular weight. The pathway for biosynthesis and interconversion of polyamines in mammalian tissues is shown in figure 1 and structures of the polyamines and intermediates in the pathway are shown in figure 2 (Pegg and McCann, 1982; Tabor and Tabor, 1984). Ornithine in tissues originates from the plasma or is syntesized from arginine by the action of arginase and is converted to putrescine via orrnithine decarboxylase, the rate-limiting enzyme of the pathway. Ornithine decarboxylase has an extremely short half-life of approximately 10 minutes. The physiological state of the animal and the presence of growth stimuli can markedly affect the turnover time of the enzyme (Janne et al., 1978).

The mechanism by which the activity of ornithine

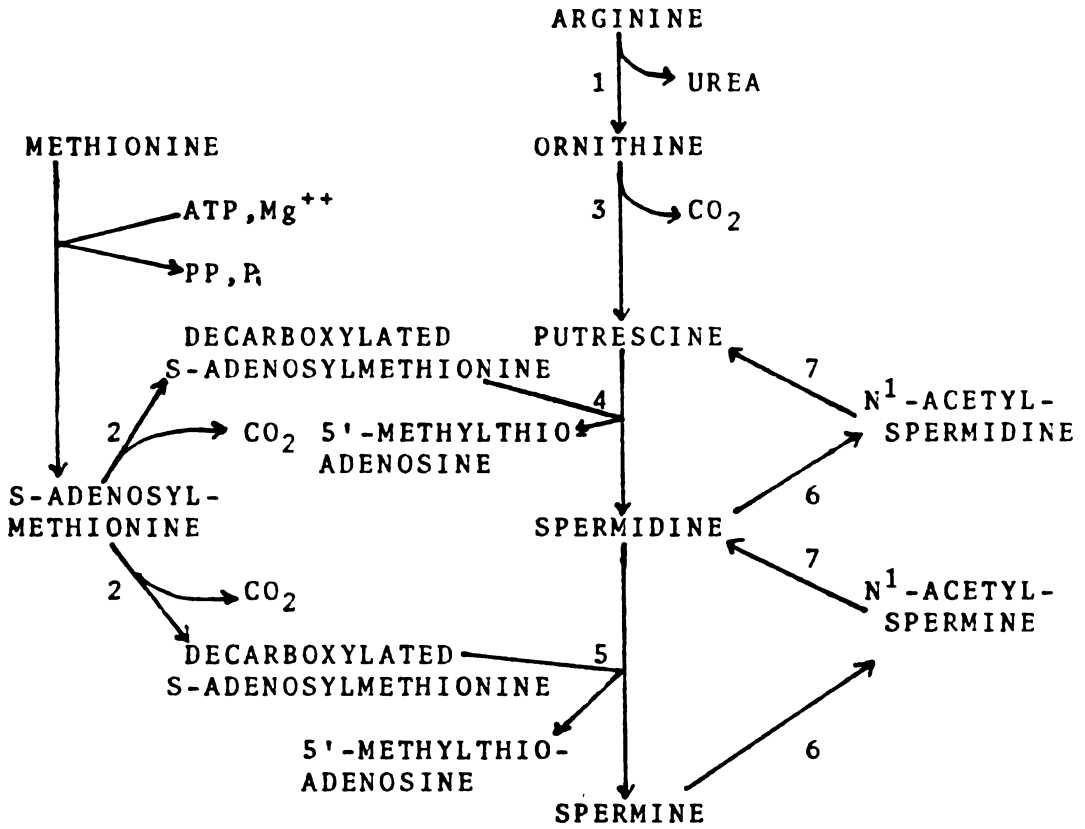


Figure 1. Pathway of polyamine synthesis and interconversion in mammalian cells. Enzymes involved are 1)arginase, 2)S-adenosylmethionine decarboxylase, 3)ornithine decarboxylase, 4)spermidine synthase, 5)spermine synthase, 6)N^1-acetyltransferase, 7)polyamine oxidase.

$H_2N(CH_2)_4NH_2$

PUTRESCINE

$H_2N(CH_2)_3NH(CH_2)_4NH_2$

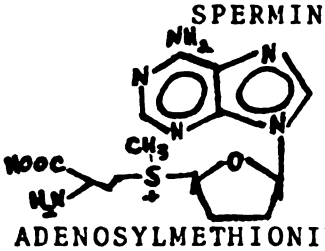
SPERMIDINE

$H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$

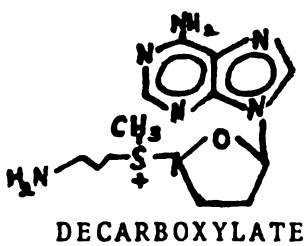
SPERMINE

$CH_3CONH(CH_2)_3NH(CH_2)_4NH_2$

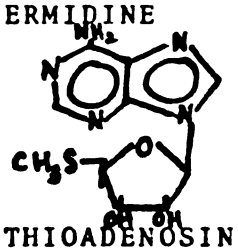
N^1-ACETYLSPERMIDINE



ADENOSYLMETHIONINE



DECARBOXYLATED ADENOSYLMETHIONINE



METHYLTHIOADENOSINE

Figure 2. Structure of polyamines and intermediates in the polyamine pathway.

decarboxylase is regulated is not clearly understood. Many possibilities have been suggested and include changes in rate of enzyme synthesis, changes in rate of enzyme degradation, presence of activators and inhibitors and posttranslational modifications of the enzyme. The posttranslational modifications involve phosphorylation-dephosphorylation of ornithine decarboxylase. The enzyme, ornithine decarboxylase, is phosphorylated by a protein kinase reaction. Spermidine and spermine have the capability to activate protein kinase while putrescine inactivates the kinase. The phosphorylated form of ornithine decarboxylase is incapable of decarboxylating ornithine to yield putrescine. This polyamine-dependent protein kinase discussed by Kuehn and Atmar (1982) behaves similarly to ornithine decarboxylase antizyme, an inhibitor of ornithine decarboxylase (Heller and Canellakis, 1981). Antizyme inactivates ornithine decarboxylase by forming a complex with the enzyme. It is not yet known whether polyamine-dependent protein kinase is ornithine decarboxylase antizyme. Glass and Gerner (1986 and 1987) present data from Chinese hamster ovary cells indicating that ornithine decarboxylase protein and subsequent activity is regulated by intracellular polyamine content through mechanisms that influence enzyme turnover. Furthermore, enhanced ornithine decarboxylase turnover requires protein synthesis. Whether polyamine-dependent protein kinase is an antizyme, a protease, or a co-factor is unknown. Fujita (1982) has found an inhibitor of

the antizyme in rat liver which is capable of reactivating the antizyme-inactivated ornithine decarboxylase. Holtta and Pohjanpelto (1986) found, in Chinese hamster ovary cells, that polyamines inhibited ornithine decarboxylase activity, but that no more than 10% of the loss of enzyme activity could be ascribed to post-translational modifications or inhibitor interaction. They found that polyamines resulted in a reduction in synthesis and an acceleration in degradation of the enzyme. Ornithine decarboxylase mRNA was unchanged suggesting that polyamines reduce ornithine decarboxylase activity by inhibiting translation. Kanamoto et al. (1986) found similiar results, i.e. changes in synthesis and degradation rates of ornithine decarboxylase in rat hepatocytes. Conversely, Kameji and Pegg (1987a and 1987b) present data indicating that polyamines regulate polyamine biosynthesis by inhibiting translation of mRNA for ornithine decarboxylase and adenosylmethionine decarboxylase.

Inhibitors of ornithine decarboxylase have been synthesized to use in studies for determining the physiological function of polyamines. Alpha-difluoromethylornithine (DFMO) is the most common inhibitor used in these studies and is believed to be an enzyme-activated irreversible specific inhibitor of ornithine decarboxylase (Metcalf et al.,1978; Pegg et al., 1987; Tyms,1986).

Conversion of putrescine to spermidine via spermidine synthase (putrescine aminopropyltransferase) requires an aminopropyl group which is derived from decarboxylated S-

adenosylmethionine. S-adenosylmethionine is decarboxylated by S-adenosylmethionine decarboxylase which is considered to be the second rate-limiting enzyme in the synthesis of polyamines (Pegg and McCann, 1982; Pegg et al., 1982). The conversion of spermidine also requires decarboxylated S-adenosylmethionine and is catalyzed by spermine synthase (spermidine aminopropyltransferase).

Adenosylmethionine decarboxylase has a half-life of approximately 1 hour. Its activity can be changed by growth stimuli, but the magnitude of change is not as great as is the change in ornithine decarboxylase activity (Tabor and Tabor, 1984). Putrescine activates and spermidine inactivates adenosylmethionine decarboxylase.

Inhibitors to adenosylmethionine decarboxylase have been used in many studies. Methylglyoxal bis (guanylhydrazone) (MGBG) inhibits the enzyme in vivo, but it is reversible and is not specific for adenosylmethionine decarboxylase (Pegg and McCann, 1982). S-(5'-Deoxy-5'-adenosyl) methylthioethylhydroxylamine (AMA), a structural analogue of decarboxylated S-adenosylmethionine, has been shown to be an irreversible inhibitor of bacterial S-adenosylmethionine decarboxylase, but is also a competitive inhibitor of bacterial ornithine decarboxylase (Paulin, 1986).

Spermidine synthase and spermine synthase are stable enzymes with long half-lives unlike the decarboxylases, therefore their quantity change very little with changes in physiological conditions (Janne et al., 1978).

Interconversion of spermine into spermidine and spermidine into putrescine occurs in vivo via spermidine N^1-acetyl-transferase (the rate-limiting enzyme of interconversion) and polyamine oxidase. The physiological significance of polyamine interconversion is believed to involve regulation of spermidine and spermine concentrations since excess spermidine and fasting induces the interconversion (Pegg et al., 1981; Seiler et al., 1981).

Degradation of putrescine can occur by the action of diamine oxidase or by the action of an enzyme that acetylates putrescine which allows it to be degraded by monoamine oxidase. Acetylation of spermidine and spermine can occur as well and may allow urinary excretion of the polyamines (Pegg and McCann, 1982).

B. FUNCTION OF POLYAMINES

1. Involvement in cellular proliferation and differentiation:

Activities of L-orthinine decarboxylase and S-adenosyl-L-methionine decarboxylase and cellular content of spermidine showed significantly high linear correlations with the specific growth rate of rat brain tumor cells when grown in culture (Heby et al., 1975). Correlation coefficients were 0.922, 0.958, and 0.951 between specific growth rate and ornithine decarboxylase activity, adenosylmethionine decarboxylase activity, and spermidine concentration, respectively. Correlation coefficients between specific growth rate and putrescine and spermine concentrations were

0.293 and -0.475, respectively. Williams-Ashman et al.(1972) found higher L-ornithine decarboxylase activities and greater putrescine concentrations in faster growing hepatomas than in slower growing hepatomas. A reduction in spermine concentration was also observed in regenerating liver (Heby and Lewan, 1971). Heby et al. (1975) suggest that reductions in spermine during times of high growth rates may be attributed to interconversion of spermine into spermidine. They also suggest that spermine synthsis may be inhibited by the accumulation of endogenous putrescine which has been demonstrated in the rat brain (Hannonen et al., 1972).

Pegg and McCann (1982) reviewed data demonstrating that in Chinese hamster ovary fibroblasts, synthesis of polyamines is initiated in the mid G_1 phase of the cell cycle, polyamines begin to accumulate in the end of G_1, and synthesis peaks as cells begin to synthesize DNA in the S phase. Putrescine, spermidine, and spermine concentrations and ornithine decarboxylase activity decrease during mid-S, begin to increase again in late S, and peak again prior to cell division. Linden et al. (1985) observed identical results in tumor cells and also observed that the decrease in ornithine decarboxylase activity coincided with the maximum ornithine decarboxylase-antizyme activity suggesting that the antizyme is involved in regulation of ornithine decarboxylase activity during the cell cycle. Addition of exogenous putrescine to cultured fibroblasts partially stimulates proliferation by shortening the S phase (Janne et al.,1978). These data suggest that polyamines may be involved in the

cell's preparation for DNA synthesis and cell division.

When quiescent cells are stimulated, amounts of ornithine decarboxylase and polyamines usually increase before increases in amounts of DNA, RNA, or protein (Tabor and Tabor, 1984). Many studies using inhibitors of polyamine synthesis confirm that polyamines are important in DNA synthesis and cell division. Mamont et al. (1976), using alpha-methylornithine, a competitive inhibitor of ornithine decarboxylase, showed in situ that in HTC cells induced to proliferate, the reduction in spermidine paralleled the inhibition of DNA synthesis and cell proliferation and that addition of polyamines reversed all effects. Similiar results were observed in many other cell types in vitro when difluoromethylornithinne or methylglyoxal bis (guanylhydrazone) were used as inhibitors of polyamine synthesis (Pegg and McCann, 1982). Inoue et al. (1981) and Kato et al. (1978) investigated the role of polyamines in regenerating rat liver in vivo and found that putrescine was required for liver regeneration and played important roles in modulation of deoxythymidine triphosphate synthesis and consequent DNA synthesis. Subsequently, Poso and Pegg (1982), using alpha-difluoromethylornithine in vivo in rats, inhibited liver regeneration following a partial hepatectomy and were able to reverse inhibition of DNA synthesis using putrescine. Steglich and Scheffler (1982) selected a mutant of Chinese hamster ovary cells that was deficient in ornithine decarboxylase which had properties very similiar to

cells treated with alpha-difluoromethylornithine, i.e. reduced ornithine decarboxylase activity, amount of putrescine, and growth rate.

In many systems, effects of polyamines on differentiation appear to be secondary to their effects on proliferation, but polyamines do directly affect differentiation in some systems. An example of such a system is milk protein synthesis in cultured mouse mammary glands. Oka et al.(1982) summarized data from their laboratory that demonstrated that spermidine is required by the mouse mammary gland for milk protein synthesis. Addition of methylglyoxal bis (guanylhydrazone), the inhibitor of S-adenosylmethionine decarboxylase, to cultured mammary cells blocked the hormonal-induced (insulin, cortisol, and prolactin) increased concentration of spermidine and subsequent milk protein synthesis. Addition of alpha-hydrazine-delta-aminovaleric acid, a specific inhibitor of ornithine decarboxylase produced similiar results. Addition of spermidine reversed the inhibition of milk protein synthesis by both inhibitors. Progesterone, an inhibitor of lactogenesis, inhibited the hormonal- induced activities of adenosylmethionine decarboxylase and spermidine synthesis, the accumulation of spermidine, and milk protein synthesis. These effects were partially reversed by cortisol, an antagonist of progesterone in the rat mammary gland. Furthermore, progesterone was more effective in inhibiting milk protein synthesis in mammary explants containing low concentrations of spermidine than in those containing high concentrations. These results

demonstrate that polyamines are important in mediating differentiation and subsequent activity of mammary cells.

Polyamines have also been shown to be important in differentiation of embryos, bone, brain cells, tumor cells, and of 3T3-L1 fibroblasts into adipocytes (Bethell and Pegg,1981; Janne et al, 1978; Pegg and McCann, 1982; Slotkin and Bartolome, 1986; Tabor and Tabor, 1984). Most of these studies were conducted using alpha-difluoromethylornithine to deplete the system of polyamine synthesis and the addition of exogenous polyamines to replenish the system in an attempt to reverse the inhibitors' effects.

Mechanisms by which polyamines affect DNA, RNA, and protein synthesis are not clearly understood. Polyamines enhance rates of replication, transcription, and translation (Pingoud et al., 1984). They have been found closely associated with DNA and RNA, are involved in stabilization of double-stranded DNA and loops in single-stranded RNA, and facilitate the condensation of nucleic acids (Cohen, 1982). In vitro studies have demonstrated polyamines having effects on association of ribosomal subunits and polypeptide chain initiation and elongation (Tabor and Tabor, 1984). Polyamines can produce organized condensed DNA structures (Gosule and Schellman, 1976) and can produce conformational changes in DNA thereby increasing the stability of DNA structures (Feuerstein et al., 1986). Ahmed et al. (1986) suggest that the cationic charge properties rather than any strict chemical structure play a role in action of polyamines

on certain protein kinase reactions because other polycationic compounds exert polyamine-like effects in the same reactions. However, inert trivalent cobalt (III) hexaamine was used as one of the cations in these experiments. Since that time, cobalt and other matal ions have been shown to induce hepatic and renal ornithine decarboxylase in rats (Yoshida et al., 1986) which would result in increased intracellular content of polyamines. Also, spermidine and spermine cannot replace the magnesium cation that is required by the kinase reactions (Ahmed et al., 1986). Chin and Sung (1972) stimulated DNA polymerase B with spermidine, but spermidine could not replace magnesium ion.

2. Involvement in intestinal growth and development:

Polyamines have received considerable attention in the gastrointestinal tract in an attempt to associate increased polyamine biosynthesis with increased mucosal growth and development. Nutritional alterations, lactation, weaning, intestinal obstructions, intestinal resections, and administration of hormones, growth factors, carcinogens, amines, and polyamines have all been shown to increase polyamine biosynthesis in the gastrointestinal tract.

Luk et al. (1980 and 1982) found that maturation of small intestinal mucosa in rats during the first 3 weeks of life was associated with increases in ornithine decarboxylase activity and putrescine, spermidine, and spermine concentrations. When alpha-difluoromethylornithine was

administered to rat pups (via the mother's milk) the increases in ornithine decarboxylase, putrescine, and spermidine were suppressed, but S-adenosylmethionine decarboxylase activity and spermine concentration were increased. The inhibitor also delayed the postnatal increase in maltase activity and the histological maturation (as measured by the number of proliferating crypt cells and villi length). Similiar results were observed in rats recovering from injury (injury was induced with arabinosylcytosine, an inhibitor of mitosis). These results indicate a significant role for polyamines in recovery from injury and maturation of intestinal mucosa of rats.

Young et al. (1984) found in lactating rats that during the first 14 days of lactation, mucosal ornithine decarboxylase increased and peaked on day 5 at the time of the maximal morphological intestinal adaptation (longest villi, deepest crypts, thicker mucosa). Difluoromethylornithine suppressed these changes suggesting that ornithine decarboxylase activity and subsequent polyamine formation is required for intestinal mucosal growth in adaptation during lactation.

Polyamines were also found to be required for the intestinal mucosa growth that occurs proximal to an intestinal obstruction and after a jejunectomy in rats (Kingsnorth et al., 1986; Luk and Baylin, 1983; Seidel et al., 1984). Kingsnorth et al. (1983) also found that difluoromethylornithine inhibited ornithine decarboxylase activity and reduced the incidence of chemically-induced

colon tumors in mice.

Maudsley et al. (1976) were able to stimulate ornithine decarboxylase up to 10-fold in the proximal small intestine of starved rats by refeeding and by insulin administration. The activity of the enzyme peaked 3 to 5 hours after insulin or refeeding and returned to basal levels by 8 hours. Most of the increased activity occurred in the duodenum and jejunum with little change in the ileum (only 2-fold). Changes in enzyme activity paralleled changes in mucosal putrescine concentrations, i.e. putrescine concentration increased 4-fold in the duodenum and jejunum, but less than 2-fold in the ileum. S-adenosylmethionine decarboxylase was stimulated only 2-fold and diamine oxidase was unchanged by refeeding.

Tabata and Johnson (1986a) administered difluoromethylornithine to rats via the drinking water and demonstrated a significant dissociation between the usual relationship of ornithine decarboxylase activity of the gastrointestinal mucosa to the growth of mucosa. Gastric mucosal growth (weight, and DNA, RNA, and protein content) was inhibited by either fasting or difluoromethylornithine and stimulated by feeding. Ornithine decarboxylase was not changed in gastric mucosa. In ileal mucosa, fasting significantly decreased mucosa weight, and DNA, RNA, and protein content and resulted in an 80% decrease in ornithine decarboxylase activity compared with normally fed rats. Refeeding fasted rats resulted in a 15-fold increase in

enzyme activity, 3-fold that of normally fed rats, and returned mucosa weight, DNA, RNA, and protein to near normal levels. Oral administration of difluoromethylornithine prevented the increase in ornithine decarboxylase, inhibited 50% of the increase in the mucosal weight, and significantly prevented the increase in DNA, RNA, and protein content associated with refeeding. Difluoromethylornithine had no significant effects on ileal mucosal parameters in normally fed rats. Effects of fasting and feeding of colonic mucosa were similiar to those of ileal mucosa, however diflouromethylornithine was less effective on colonic mucosa.

These results suggest that 1)polyamines are necessary for growth of gastrointestinal mucosa following refeeding in fasted rats, 2)"mucosal" ornithine decarboxylase may not be responsible for the synthesis of polyamines for gastrointestinal growth, and 3)the polyamines necessary for growth may not be synthesized in the mucosa. Tabata and Johnson (1986a) suggest that the blood and/or the gut contents may serve as a source of polyamines. Polyamines in the blood may originate from the liver and kidney, which both increase polyamine synthesis after refeeding (Moore and Swenseid, 1983) whereas polyamines in the gut contents may originate from bacterial decarboxylation as suggested by Tabata and Johnson (1986a). Oral administration of antibiotics to rats reduced microfloral ornithine decarboxylase and reduced the increases in colonic mucosal DNA, RNA, and protein concentrations after colonic ligation (Osborne and Seidel, 1987). This indicates that polyamines

produced by gastrointestinal microflora are used by intestinal mucosa. Tabata and Johnson (1986a) point out that if uptake of luminal amines explain resistance of ileal and colonic mucosa to difluoromethylornithine then the tissues should exhibit more resistance in fed rats, which was the case in their study.

Seidel (1986) conducted studies examining polyamine biosynthesis and gastrointestinal growth in the duodenum, jejunum, and ileum. Ornithine decarboxylase activity was extemely low in the duodenum, jejunum, and ileum mucosa of fasted rats. In rats fed ad-libitum enzyme activity was similiar to fasted rats in the duodenum, but increased distally to reach levels 200-fold above fasted levels in the ileum. Difluoromethylornithine in the drinking water of ad lib fed rats reduced enzyme activity in both proximal and distal sections of the small intestine. These changes were associated with no change in duodenal mucosal DNA, RNA, and protein contents and significantly lower ileal mucosal DNA, RNA, and protein contents. These results suggest that duodenal ornithine decarboxylase activity is regulated by factors other than the presence of food in the gut (Seidel, 1986). Perhaps pancreas secretions may contain polyamines. Rats fed raw soybean flour had increased amounts of putrescine and spermidine in their pancreas (Loser et al., 1987).

Tabata and Johnson (1986b) used the Thiry-Vella rat model to determine whether stimulation of ornithine

decarboxylase is mediated directly or humorally. Ornithine decarboxylase activity increased 40-fold in mucosa of intact jejunum and 4-fold in Thiry-Vella jejunal mucosa following refeeding of fasted rats. Infusion of intestinal contents from fed rats into Thiry-Vella loops stimulated their enzyme activity 10-fold indicating that meal-induced mucosal ornithine decarboxylase is mediated via both humoral and direct mechanisms. They further examined the role of the gut contents in stimulating the enzyme and found that if the infusate was lyophilized, to remove dietary amines (dietary amines are volatile under alkaline conditions and thus can be removed by lyophilization; Lichtenberger et al., 1982), there was no change in enzyme activity. Furthermore, addition of dimethylamine to the lyophilized infusate produced identical results to the normal infused gut contents (i.e. stimulated ornithine decarboxylase activity to the same degree). This suggests that dietary amines are one constituent of luminal contents which are responsible for activation of intestinal mucosal ornithine decarboxylase. Dietary casein, gastrically intubated amino acid mixtures, and individual dietary amino acids have also been shown to stimulate ornithine decarboxylase activity (Fujimoto et al., 1978; Minami et al., 1985; Moore and Swendseid, 1983).

Seidel (1986) conducted studies to determine if the presence of food in the gut may be stimulating ornithine decarboxylase activity via hormones. He presented direct evidence for hormonal involvement in rats that had undergone ileal bypass surgery. Refeeding after surgery resulted in a

significant increase in ornithine decarboxylase activity in both the intact intestinal segment and the bypassed segment indicating that postprandial induction of the enzyme is mediated via some hormone(s).

Refeeding carbohydrate alone (dextrose) to fasted rats was sufficient for maximal ornithine decarboxylase induction and comparable to complete balanced diets, whereas mixed amino acids or triglycerides only slightly induced the enzyme and were no more effective than were nonnutritive solutions of saccharin or cellulose (Seidel, 1986). This suggests that hormone(s) involved in induction of ornithine decarboxylase is one which is released in response to carbohydrate digestion. Why the amino acid mixtures fed by Seidel (1986) only slightly stimulated ornithine decarboxylase activity whereas Moore and Swenseid (1983) found amino acid mixtures to be extemely effective inducers of the enzyme may be a result of the route of administration; the latter authors gastrically intubated the mixtures. Maudsley et al. (1976) have demonstrated that subcutaneous insulin administration has induced ornithine decarboxylase in the small intestinal mucosa of starved rats which suggests that insulin may be a candidate for inducing ornithine decarboxylase. Seidel (1986) speculated that the gut peptides, particularly enteroglucagon, may be the mediator. Enteroglucagon is released in response to dietary carbohydrates (Walsh, 1981) and is involved in proliferation of the ileal mucosa (Magee and Dalley, 1986). This may explain results of Seidel's

studies (1986) in which the presence of food in the gut induced ornithine decarboxylase activity more in the distal sections than in the proximal sections of the small intestine. Dowling and Miazza (1985) proposed that enteroglucagon binds to cell receptors which trigger intracellular cascades involving cAMP and polyamines as second messengers. Inhibition of phosphodiesterases by treatment with isobutylmethylxanthine resulted in induction of ornithine decarboxylase activity in mucosa of the jejunum, ileum, and colon, but not in the duodenum, which suggests that jejunal, ileal, and colonic ornithine decarboxylase may be regulated by a hormone that uses cAMP as a second messenger (Seidel,1986).

Fitzpatrick et al. (1987) found that intraperitoneal administration of epidermal growth factor (EGF) to fasted rats significantly increased ornithine decarboxylase activity in isolated enterocytes from the villus tip, midvillus, and crypt fractions and was greatest in the villus tips of the ileum. An increase in S-adenosylmethionine decarboxylase also occurred in midvillus enterocytes and was also greatest in the ileum. The EGF which was administered intragastrically did not induce enzyme activities suggesting that EGF-uragastrone receptors are present only on the serosal surface of enterocytes (Fitzpatrick et al., 1987). The lack of enzyme induction after intragastric administration of EGF could be due to hydrolysis of EGF from proteases, however, Thornberg et al. (1984) found that 15% and 38% of labelled EGF was present in the intestinal wall

and contents, respectively, after oral administration to suckling rats. These results appear contradictory to those of Dembinski et al. (1982) who were able to stimulate duodenal mucosal DNA synthesis and concentration by EGF administered intraperitoneally or intragastrically, however, DNA synthesis after intragastric administration of EGF may not depend on induced ornithine decarboxylase activity.

Dembinski et al. (1984) demonstrated that dietary amines may directly stimulate gastrointestinal mucosa growth. Rats which were antrectomized (partial removal of the glandular stomach) had reductions in weight and DNA, RNA, and protein content of the oxyntic gland portion of the stomach, duodenum, jejunum, and colon and in serum gastrin. Feeding dimethylamine in the diet (100 umoles/g food) prevented these differences in the oxyntic gland and duodenal mucosa with a smaller but still significant increase in the more distal sections. Amines had no effect on serum gastrin suggesting direct effects of amines on growth.

Subsequently, Seidel et al. (1985) infused ethylamine or putrescine (1 umole/h for 66h) into the ileal lumen of fasted rats to determine the effect of amines on intestinal mucosal growth and polyamine biosynthesis. Both amines were accompanied by ileal mucosal growth as measured by total mucosal RNA, DNA, and protein in the 2 cm segment of mucosa surrounding the infusion catheter tip when compared to saline-treated animals. Growth was also stimulated by amines proximal and distal to the catheter tip. Putrescine induced

ornithine decarboxylase 2-fold and S-adenosylmethionine decarboxylase 10-fold while ethylamine induced the activities 6-fold and 5-fold, respectively, in mucosa at the catheter tip. Amines had no significant effect on tissue concentrations of individual polyamines and no effect on serum gastrin concentration. Only total tissue concentration of polyamines were measured in this experiment. If extracellular and intracellular concentrations had been measured, Seidel et al. (1986) assume that the extracellular concentrations would have been elevated in the putrescine-treated rats which would agree with investigations of Mamont and Danzin (1981) in that extracellular putrescine induces S-adenosylmethionine. High concentrations of liver putrescine in vitro have inhibited ornithine decarboxylase activity in liver (Pegg et al.,1978), but in other similar studies high cocentrations of putrescine have been associated with high activities of ornithine decarboxylase (Janne etal.,1978). Since ethylamine is not known to substitute for putrescine in the polyamine biosynthetic pathway, Seidel et al.(1985) speculates that ethylamine acts at the plasma membrane of enterocytes to change polyamine biosynthetic enzyme activities in an inhibitory or stimulatory fashion. They also speculate that putrescine may act similarly, however, putrescine has been demonstrated to be actively taken up by enterocytes via a sodium-dependent, specific mechanism which is more prevalent in the distal intestine (Kumagai and Johnson, 1987) suggesting that uptake of putrescine by enterocytes or mediation at the plasma membrane or both may

affect polyamine biosynthesis and the gastrointestinal mucosal growth.

Several studies have demonstrated roles for ornithine decarboxylase and polyamines in differentiation of enterocytes as well. Baylin et al. (1978) found in rat small intestinal mucosa that ornithine decarboxylase activity was much greater in the mature cells of the villus tip region than in the proliferating cells in the crypt region suggesting that polyamine biosynthesis may be more important in protein synthetic activities of mature cells rather than in prolferative activity of the crypt cells. Sepulveda et al. (1982) found similar results in rats fed low protein diets. Rats fed low protein diets had lower proliferation rates (measured by incorporation of tritiated thymidine), but had higher ornithine decarboxylase activities in isolated enterocytes when compared to rats fed high protein diets. Ornithine decarboxylase was associated with differentiating villus cells in the low protein group, but was uniformly distributed along the villus/crypt axis in the high protein group. They hypothesized that when rats receive low protein diets ornithine decarboxylase plays a larger role in differentiating the enterocytes into absorptive cells and a smaller role in proliferation of enterocytes.

Fitzpatrick et al. (1986) stimulated ornithine decarboxylase and S-adenosylmethionine decarboxylase activities in enterocytes of villus tips and midvillus fractions of enterocytes by refeeding fasted rats. No change in enzyme activities occurred in crypt cells. Putrescine

concentration was increased in midvillus enterocytes and in crypt cells, but not in enterocytes of villus tips. This indicates that in villus cells putrescine is synthesized from ornithine via ornithine decarboxylase, whereas in crypt cells putrescine may not be synthesized, but may be taken from the extracellular compartment. Putrescine in the extracellular compartment may arise through secretions from villus cells, as suggested by Fitzpatrick et al.(1986), or from luminal contents as suggested by Tabata and Johnson (1986) and be taken up by crypt cells via a mechanism similiar to one demonstrated by Kumagai and Johnson (1987). If crypt cells are synthesizing putrescine they may be doing so via interconversion of spermidine into putrescine by spermidine N^1-acetyltransferase. Spermidine concentration in crypt cells of refed rats was 53% of the fasting concentrations in the study of Fitzpatrick et al. (1986) suggesting that spermidine could be a source of putrescine in crypt cells.

Shinki et al. (1986) found opposite observations in chick duodenal mucosal in that ornithine decarboxylase activity was greater in crypt cells and N^1-acetyltransferase activity greater in villus cells of both vitamin D- deficient and 1-alpha, 25-dihydroxyvitamin D_3-supplemented chicks. Putrescine, spermidine, and spermine were also more concentrated in crypt cells than in villus cells. The increase of duodenal putrescine by the vitamin coincided quantitatively with the amount of putrescine synthesized from spermidine, but not from ornithine indicating that spermidine

N^1-acetyltransferase had a larger role than ornithine decarboxylase in the increase of putrescine synthesis in this situation.

Summary of the Literature Review

The literature reviewed has demonstrated that partial substitution of milk protein in milk replacers for young piglets and preruminant calves often results in below normal animal performance. Reduced performance in neonates receiving soybean proteins may be due to one or a combination of factors, such as increased passage of digesta through the gastrointestinal tract, reduced nutrient digestion and/or absorption, and intestinal allergic reactions. These factors have been associated with the presence of components such as soybean trypsin inhibitors, allergens, lectins, and inadequately identified factors as well as the absence of some other components, e.g. methionine.

Polyamines are required for rat intestinal mucosal growth and developement, maturation, adaptation, and recovery from injury. Inhibitors preventing the biosynthesis of these polyamines have prevented these growth responses, whereas addition of putrescine and other amines (dimethylamine or ethylamine) to rat diets or directly to the small intestine can promote intestinal mucosal growth. These growth responses include increases or decreases in intestinal epithelium renewal rate, villus length, crypt depth, digestive enzyme concentrations, RNA, DNA, and protein concentrations, and polyamine biosynthetic enzyme

concentrations.

Soybean proteins are detrimental to some of the small intestinal parameters listed above. Inclusion of soybean protein in liquid diets of neonates affects the villus and crypt cell population. The exact mechanism of how this occurs is speculative. One idea obtained from reviewing existing reports is that diets containing soybean proteins may be improved if supplemented with amines and that some soybean proteins may contain components that either directly or indirectly reduce the biosynthesis of polyamines in the small intestinal epithelium which in turn affects intestinal proliferation and differentiation. This idea has not been pursued and appears worthy of investigation. Hence, the following studies were conducted in piglets and calves to examine the effects of 1) soybean proteins on the growth, development, and function of the small intestine and 2) dietary amines supplementing these soybean proteins on small intestinal variables.

MATERIALS AND METHODS

Experiment 1

Sixteen Holstein bull calves were obtained from local dairy farms or the Michigan State University dairy farm and placed in outdoor, straw-bedded, individual hutches at the university dairy farm. Calves were fed liquid diets twice daily via nipple bottles. Amount of diet fed daily was equal to 8% of the body weight. Calves received colostrum and whole milk from birth through day 3 of age and one of four liquid milk replacers (12% dry matter) from day 4 through day 21 of age. Milk replacer diets were 1) all-milk protein (AMP) (prepared by Milk Specialties Co.-Dundee,IL), 2)diet 1 with 20% of the protein replaced with soybean protein concentrate (SPC) (prepared by Milk Specialties Co.-Dundee, IL), 3) diet 2 supplemented with putrescine dihydrochloride (Sigma Chemical Co.-St. Louis, MO), at 25g (or 0.16 moles)/kg diet dry matter (SPP), and 4) diet 2 supplemented with ethylamine hydrochloride (Aldrich Chemical Co.-Milwaukee, WI) at 25g (or 0.31 moles)/kg diet dry matter (SPE). Composition of diets 1 and 2 are shown in table 1.

Body weights of the calves were recorded twice weekly and rectal temperatures were recorded at least every other day. Physical appearance of the feces was scored by the

Table 1. Composition of diets 1 and 2 used in experiment 1.

Ingredients[a], %	AMP	SPC
Spray dried whey	34.0	51.0
Whey protein concentrate/ skimmed milk	38.0	5.5
Delactosed whey	7.0	7.0
Soy protein concentrate	0.0	15.0
Fat	20.0	20.0
Premix, mineral and vitamin	1.0	2.5
Nutrient Composition		
Crude protein, %	20.00	21.00
Energy, ME, kcal/kg	4161.63	3981.15
Crude fiber, %	0.00	0.50
Fat, %	20.00	20.00
Ash, %	7.42	6.87

[a]Medicated with 55 g chlortetrcycline/1000 kg.

guidelines of Larson et al. (1977) into four categories: normal (code 1)=firm but not hard, original form is distorted slightly after dropping to floor and settling; soft (code 2)=does not hold form, piles but spreads slightly (i.e., soft serve ice milk); runny (code 3)=spreads readily to about 6mm depth (i.e., pancake batter); watery (code 4)= liquid consistency, spatters (i.e., orange juice).

On days 5, 12, and 19 of age, small intestinal absorption was evaluated by xylose absorption tests (Merritt and Reed, 1980). A 10% xylose solution was administered via a nipple bottle (0.5g xylose/kg body weight) 8 hours after the AM feeding. Ten ml of blood were collected in heparinized tubes from the jugular vein at 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 hours after the administration of the xylose. The blood was centrifuged and plasma was frozen at -20 C. Plasma xylose was determined later by the orcinol method (Bolton et al., 1976). Details are in Appendix A.

On days 7, 14, and 21 of age, segments of the proximal and distal jejunum (sites 1 and 2, respectively) were surgically excised 12 to 15 hours after the PM feeding. Anesthesia was acheived by intramuscular administration of a Xylazine-Ketamine mixture. Xylazine (Rompun-Haver, Shawnee, KS) and Ketamine (Vetalar-Parke Davis, Morris Plains, NJ) were mixed in the same syringe and administered at 0.1 mg/kg and 5mg/kg, respectively. Subsequently, a paravertebral nerve block was performed by injecting 2 ml of 2% licocaine (Vedco, Inc.-Overland Park, KA) around the last thoracic

nerve (T13 and first two lumbar nerves (L1, L2,). The calves were recumbant within 3 minutes of administering the Xylazine-Ketamine mixture and they were placed in left lateral recumbency on a padded surgical table.

The right paralumbar fossa and flank areas were clipped and prepared for aseptic surgery. A 6- to 8- cm dorsoventral skin incision was made in the right paralumbar fossa beginning 4-cm ventral to the transverse process of the third lumbar vertebrae. The external and internal abdominal oblique muscles were incised seperately by sharp dissection. Metzenbaum scissors were used to incise the transverse abdominus muscle peritoneum. Muscle bleeding was controlled by ligation. The cecal apex and terminal portion of the small intestine (ileum) were located and exteriorized. The ileocecocolic junction and the ileocecal fold were identified and used as reference points.

The following procedures are described for obtaining intestinal segments in the proximal and distal regions of the jejunum (sites 1 and 2, respectively). The intestinal segment to be resected was isolated and the remainder of the intestinal tract returned to the abdomen. The isolated segment was packed off with saline moistened sponges in an attempt to prevent contamination and dehydration. The contents within the lumen of the isolated segment were milked away from the intended surgical site. The mesenteric vessels supplying the intestinal segment were isolated and ligated. An assistant held the intestine between the thumb and forefingers. A number 10 scalpel blade was used to excise a

4-cm segment of gut and the accompanying mesentery. Moistened gauze sponges were used to rid the cut surfaces of intestinal contents and blood.

An end-to-end anastomosis was performed using 4-0 intestinal monofilament polydioxanone (PDS). The ends of the bowel were approximated with 10-to-12 simple interrupted through-and-through sutures. The first and second suture were placed at the mesenteric and antimesenteric borders, respectively. This was done to divide the suture planes in half which permitted better approximation of the exposed ends. Particular attention was given to preventing protrusion of the mucosa, which if exposed would increase the chances of adhesions forming. Following completion of the anastomosis, the defect in the mesentery was sutured with 3-0 polyglycolic acid. The anastomosis was examined for leakage. The surgical area was lavaged and the bowel returned to the abdominal cavity. The abdominal cavity was lavaged with a saline solution containing 200 mg of gentamycin sulfate/L (Gentocin, Schering Corp., Kenilworth, NJ). A two layer closure was performed in closing the abominal wall. The peritoneum and transverse abdominal muscles were not sutured closed. Number 1 polyglycolic acid, in a simple continuous pattern, was used to close the internal and external abdominal oblique muscles as a single layer. The skin incision was closed with 1 monofilament polypropylene (prolene) in a Ford interlocking pattern. Each calf was given 50 mg of banamine (Flunixin meglumine-Schering Corp.,

Kenilworth, NJ) immediately after surgery and 12 hours later as an analgesic. One gram of ampicillin (Omnipen-N, Wyeth Lab., Phila., PA) and 80 mg of gentamycin sulfate were given intramuscularly 2 times a day for 3 days. Surgery times ranged from 90 to 120 minutes.

Seven and fourteen days after the intitial surgery, the same calves were prepared for surgery using the identical presurgical preparations and anesthesia as described previously. After entering the abdomen through the previous surgical incision, the previous anastomotic sites were located. The second and third intestinal segments were taken 15 to 20 cm proximal to the previous anastomosis. The intesinal resection, abdominal wall closure, and postoperative care was performed as described. After obtaining the 21 day segments, the calves were euthanized by intravenous administration of T-61 Euthanasia Solution (Hoechst- Somerville, N.J.).

Immediately after excising the intestinal segment from the calf, the segment was flushed with ice-cold physiological saline solution (0.9% NaCl) and blotted dry with a paper towel. One-third of the segment was cut off with a blade and placed in a 10% solution of buffered phosphate-formalin and later used for histological measurements. Briefly, part of the specimen was imbedded in paraffin and at least four 5-um sections were mounted on a slide and stained with eosin and hematoxylin. The sections were sliced along the length of the villi so that cross sections of villus-crypt regions were formed. Sections were examined under a phase-contrast

microscope at 25X magnification to measure the mitotic index (Hooper, 1961). Mitotic index, a measurement of the rate of intestinal epithelium proliferation, was calculated as the percentage of crypt enterocytes undergoing mitotic divisions. At least 500 crypt enterocytes were counted in regions where individual crypts and villi were continuous.

Another portion of the formalin-fixed tissue was stained with New Methylene Blue and examined under a dissecting microscope equipped with an ocular micrometer at 30X magnification to measure villus length and crypt depth. Ten villi and crypts were measured on each section and average villus heights and crypt depths were calculated.

The mucosa was scraped from the remaining segment and divided into three pieces. One piece of mucosa was placed in ice-cold 50mM sodium phosphate buffer (glycerol 14%, pH 7.2) and homogenized with a Brinkman polytron. Supernatants for protein and enzyme determinations were obtained by differential centrifugation. Lactase activity was determined by the glucose oxidase method of Dahlqvist (1964) and Messer and Dahlqvist (1966); peptidase activity by the amino acid oxidase method of Nicholson and Kim (1975); ornithine decarboxylase activity (Slotkin and Bartolome, 1983); and protein with a protein assay kit (BioRad Laboratories, Calif.) which utilizes the Bradford procedure (Bradford, 1976). All supernatants were stored at -20 degrees C with the exception of that for the determination of ornithine decarboxylase activity which was stored at -85 degrees C.

Sample preparation and assay procedures are described in more detail in the appendix. A second piece of mucosa was placed into 10% trichloroacetic acid (TCA) homogenized with a Brinkman polytron, and centrifuged at 26,000xg for 10 minutes. The TCA was removed from the supernatant with diethylether (at least 3 extractions) and the aqueous supernatant was frozen at -20 degrees C until analized for concentrations of putrescine, spermidine and spermine. Polyamine concentrations were determined by high-performance liquid chromatography (modification of Brown et al., 1986) (See appendix for detailed procedures). Ratios between the three polyamines were calculated.

A third piece of mucosa was frozen in a sealed vial at -20 degrees C and later used for the determination of DNA and RNA concentrations, by the diphenylamine and orcinal methods, respectively (Munro and Fleck, 1966 and Tseng and Johnson, 1986), and protein concentration (Bradford, 1976 and Tseng and Johnson, 1986) (see appendix for detailed procedures).

Prior to laboratory analyses all samples were blocked so that each assay run included samples from all treatments, sites, and days in an attempt to evenly distribute any assay variation across all treatments, sites, and days.

Small intestinal histology, enzyme acivity, and metabolite data were statisically analyzed by least squares analysis of variance of a double split-plot in space and time design (Gill, 1978). Weekly least square means of rectal temperatures, fecal scores, and weight gains and of peak plasma xylose concentrations, times of peaks, and

coefficients of regression equation variables generated from xylose absorption curves were statistically analyzed by least squares analysis of variance of a split-plot with repeated measurement design (Gill,1978). Least square means of total experimental period weight gains were statistically analyzed by one-way analysis of variance (Gill,1978). Least square means were generated by the General Linear Models (GLM) procedure of the Statistical Analysis System (SAS, 1982).

The double split-plot in space and time model was:

$$y_{ijkl} = u + \alpha_i + D_{(i)j} + \beta_k + (\alpha\beta)_{ik} + (D\beta)_{(i)jk} + \gamma_l + (\alpha\gamma)_{il} + (\beta\gamma)_{kl} + (\alpha\beta\gamma)_{ikl} + E_{(ijkl)}$$

y_{ijkl} = observed variable.
u = overall mean.
α_i = fixed effect of ith treatment, i = 1,2,3,4.
$D_{(i)j}$ = animal within treatment (error for testing treatments), j = 1,2,3,4.
β_k = fixed effect of kth site, k = 1,2.
γ_l = fixed effect of lth day, l = 1,2,3.
$(\alpha\beta)_{ik}$, $(\alpha\gamma)_{il}$, $(\beta\gamma)_{kl}$, and $(\alpha\beta\gamma)_{ilk}$ = interactions between main effects.
$E_{(ijkl)}$ = residual error.
$(D\beta)_{(i)jk}$ = error for testing site and interaction of trt. with site.

The split-plot with repeated measurement model was:

$$y_{ijk} = u + \alpha_i + D_{(i)j} + \beta_k + (\alpha\beta)_{ik} + E_{(ijkl)}$$

y_{ijk} = observed variable.
u = overall mean.
α_i = fixed effect of ith treatment, i = 1,2,3,4.
$D_{(i)j}$ = animal within treatment (error for testing treatments), j = 1,2,3,4.
β_k = fixed effect of kth day (or week), k = 1,2,3.
$(\alpha\beta)_{ik}$ = interaction between treatment and day.
$E_{(ijk)}$ = residual error.

The one-way analysis of variance model was:

$yij = u + \alpha: + E_{(i)j}$

yij = observed variable.
u = overall mean.
αi = fixed effect of ith treatment, i = 1,2,3,4.
$E_{(i)j}$ = residual error.

Quadratic regression equations were generated from the xylose absorption curves using the following model:

$Y = B_0 + B_1X + B_2X^2 + E$

Y = plasma xylose.
X = time.
B_0 = origin (extrapolated value of Y when X is fixed at 0).
B_1 = coefficient of X variable.
B_2 = coefficient of X^2 variable.
E = random error of relationship.

Statistical contrasts among least square means of treatments, sites, and days were performed using Bonferroni t statistics (Gill,1978). Treatment nonorthogonal contrasts were 1 vs 2, 1 vs 3 and 4, and 2 vs 3 and 4. Other contrasts were performed using Scheffe's test (Gill, 1978).

Experiment 2

Six litters of crossbred piglets were obtained from the Michigan State University swine farm. Eight piglets per litter were taken from the sow at 2 days of age, paired, and

housed in elevated, mesh wire-bottomed, stainless steel pens. Piglets were weighed when taken from the sow and daily thereafter. Pairs were randomly assigned to one of the four dietary treatments. Piglets in all treatments received a liquid replacer diet reconstituted to 15% dry matter with tap water just before feeding. Treatment 1 was an all-milk protein milk replacer diet (AMP) (Chore-Time Equipment, Inc.-Milford, Indiana). Treatment 2 was diet 1 with 20% of the protein replaced with a specially-processed soybean protein isolate (SPI) (prepared by Milk Specialties Co.-Dundee, IL). Treatment 3 was diet 2 supplemented with putrescine dihydrochloride (Sigma Chemical Co.-St. Louis, MO) at 25 g (or 0.16 moles)/kg diet dry matter (SPP). Treatment 4 was diet 2 supplemented with ethylamine hydrochloride (from Aldrich Chemical Co.-Milwaukee, WI) at 25g/kg diet dry matter (SPE). Composition of diets 1 and 2 are shown in table 2. Amount of diet fed daily was equal to 30 g dry matter/kg body weight$^{\cdot 75}$ on day 3 of age and was increased to 50 g dry matter/kg body weight$^{\cdot 75}$ by day 5. Piglets were fed four times per day via stomach tubes attached to plastic syringes.

On day 5 of age, small intestinal absorption as quantitated by xylose absorptive tests (Merritt and Reed, 1980) in all piglets. A 10% xylose solution was administered via a stomach tube (0.5g xylose/kg body weight) after a 12 hour fast. About three ml of blood were collected from the vena cava in heparinized tubes at 0, 2 and 3 hours after xylose administration. Blood was processed and plasma

Table 2. Composition of diets 1 and 2 used in experiment 2.

Crude protein, %	17.0
Crude fat, %	15.5
Crude fiber, %	0.2
Vitamin A, I.U./kg	11,000
Vitamin D_3, I.U./kg	2,750
Vitamin E, I.U./kg	22
Oxytetracycline, g/1000 kg	110
Neomycin base, g/1000 kg	77

analyzed as it was previously described for experiment 1.

On day 7 of age, one-half of the piglets (one piglet from each pair) were anesthesized by intramuscular administrations of Azaperone (Stresnil-Pitman-Moore, Inc., Washington Crossing, NJ) and Ketamine (Vetalar- Parke-Davis, Morris Plains, NJ). Azaperome and Ketamine were administered at 4.4mg/kg and 8.8mg/kg, respectively, six hours after feeding. The abdominal cavity was open and three segments (each approximately 4 cm long) of the small intestine were excised and processed as in experiment 1, except that the supernatant obtained for the determination lactase activity was used for the determination of sucrase activity by the glucose oxidase method of Dahlqvist (1964) and Messer and Dahlqvist (1966 appendix A). The duodenum sample (site 1) was obtained from the first 4 cm of the small intestine immediately distal to the pylorus, the jejunum sample was obtained 60 cm proximal to the ileocecolic junction (site 2), and the ileum sample was obtained from the last four cm of the small intestine immediately proximal to the ileocecolic junction (site 3). Three milliliters of jejunal contents were collected, placed in 3 ml of 10% trichloroacetic acid solution, and processed along with the tissue samples for polyamine determination (Brown et al., 1986). Piglets were sacrificed by an intravenous administration of T-61 Euthanasia Solution (Hoechst, Somerville, NJ) after all intestinal segments were excised. The remaining piglets were given xylose absorption tests on day 12 of age and

anesthesized on day 14 of age for collection of tissue and luminal contents. Tissue and luminal contents were processed as previously described for the day 7 samples.

Before assaying, all samples were blocked as in experiment 1 to minimize assay variation. Statistical analysis was performed using the models previously described in experiment 1.

Experiment 3

Eight crossbred piglet littermates were obtained from the Michigan State University swine farm. Piglets were taken from the sow at 2 days of age and randomly assigned to one of the two dietary treatments. Treatment 1 was diet 2 of experiment 2 which was supplemented with soybean protein isolate (ICN Biochemicals-Costa Mesa, CA; 90% crude protein) at 111.1g/kg diet dry matter (SPI). Treatment 2 was the above diet supplemented with putrescine dihydrochloride (Sigma Chemical Co.-St. Louis, MO) at 25g(or 0.16 moles)/kg diet dry matter (SPP). Final crude protein content of the diets was 27%. Amount fed and method of feeding was the same as in experiment 2. Body weights were recorded when the piglets were taken from the sow and daily thereafter.

On day 5 of age, all piglets were given xylose absorption tests as described in experiment 2.

On day 7 of age, all piglets were anesthesized to obtain segments of the small intestine. Segments were excised, processed, and analyzed as described in experiment 2.

Data was statistically analyzed by analysis of variance

of a split-plot with repeated measurement design and by one-way analysis of variance (Gill, 1978) as described in experiment 2.

RESULTS AND DISCUSSION

Experiment 1

General observations:

The surgical procedures conducted on the calves were very successful. Out of a total of forty-eight surgeries only three led to complications. Three calves (#104, 114, and 115) died within five days after their day-7 surgery. Postmortem examination revealed that these three calves died from ileus. This was probably a result of the trauma induced by over-manipulating the small intestine during surgery. All other calves fully recovered from surgeries and consumed feed within 3 to 4 hours after surgeries. Previous intestinal excision sites were examined during the day 14 and 21 surgeries and all appeared normal with no swelling. The three calves which died during the experiment were deleted from statistical analysis.

The health of the calves which completed the entire experiment was excellent. Fecal scores and rectal temperatures were normal for all calves throughout the experimental period (Table 3). Fecal scores and rectal temperatures were not statistically different among calves fed different diets, but fecal score increased (feces became softer) with age ($p<.002$). Average body weight gain over the

Table 3. Fecal scores, rectal temperatures, body weight gains, and blood packed cell volumes of calves fed four diets.

	Age	Diet[g]				Weekly	
	(weeks)	AMP	SPC	SPP	SPE	mean	SEM
Fecal score, 1 to 4[ae]	1	1.2	1.0	1.1	1.3	1.2	0.08
	2	1.6	1.6	2.0	1.3	1.6	0.08
	3	1.5	1.5	1.8	1.5	1.6	0.08
Treatment mean		1.4	1.4	1.6	1.4		
SEM[h]		0.1	0.1	0.1	0.1		
Rectal temperature, °C	1	39.3	38.8	38.7	39.0	39.0	0.06
	2	39.1	39.1	39.0	39.1	39.0	0.06
	3	39.1	38.8	39.0	39.1	39.0	0.06
Treatment mean		39.2	38.9	38.9	39.1		
SEM		0.06	0.09	0.07	0.07		
Body weight gain, g	1[b]	136	-272	681	-272	68	229
	2[c]	409	681	136	363	404	229
	3[d]	545	409	817	91	482	229
SEM		281	397	324	324		
Packed cell volume, %[f]		44.8	51.3	23.0	45.9		
SEM		10.8	17.0	17.0	8.5		

[a]1=firm; 4=watery
[b]1=total gain from week 1 to 2.
[c]2=total gain from week 2 to 3.
[d]3=total gain from week 1 to 3.
[e]Different among weeks ($p<.002$).
[f]Packed cell volumes are treatment means for 2 to 3 calves per diet group from blood obtained for plasma xylose anaysis.
[g]AMP=all-milk protein milk replacer; SPC=AMP with 20% of the protein replaced with soybean protein concentrate; SPP=SPC plus putrescine; SPE=SPC plus ethylamine.
SEM=standard error of the mean.

3 week experimental period was 482 g and was not different among calves receiving different diets (Table 3). Average gain from the first to second week of age tended to be less than that from the second to third week of age for AMP, SPC, and SPE. Gain tended to be greatest for calves fed SPP. Gain may have been greater if greater amounts of milk replacer had been fed (e.g. 10% of body weight, rather than 8%, Appleman and Owen, 1975). Total 3 week gain in this experiment are greater than those reported in a study (Silva, 1984) in which calves received all-milk protein milk replacers or milk replacers containing 66% of the protein from soybean protein concentrate (482 g vs -740 g).

Packed cell volumes obtained from blood collected during xylose absorption tests were not different, but tended to be less for blood from calves fed SPP (Table 3). This was investigated further by submitting blood samples from calf #113 (AMP) and calf #116 (SPE) to the Michigan State University Veterinary Clinical Center Pathology Laboratory. Blood from calf #116 was reported to have a lower packed cell volume, % red blood cell, and hemoglobin concentration than blood from calf #113, however values for these parameters were in the normal ranges for the calves of these ages (Table 4). Only limited conclusions can be made from two hematology reports and the data on packed cell volume. However, Ballas et al. (1985) reported that polyamines interfered with hemoglobin synthesis in vitro; high concentrations of putrescine (>10mM) reduced synthesis of hemoglobin. The

Table 4. Packed cell volume and red blood cell and hemoglobin concentrations of blood from two calves fed two diets.

	Diet[a]	
	AMP	SPE
Packed cell volume, %	35	25
Red blood cell, $x10^6$/ul	9.32	6.24
Hemoglobin, g/dl	11.4	8.2

[a]Calf #113 fed AMP; Calf #116 fed SPE.

effects of amine supplementation on hematology variables should be examined in any future trials.

Absorption measurements:

Xylose absorption curves for diets 1, 2, 3, and 4 are shown in figure 3. Since zero hour plasma xylose concentrations were not different among diets (mean zero hour value was 16.6 mg/dl), the zero hour value was subtracted from all plasma values so that all curves intercepted the origin. The increase in plasma xylose from 0 to 1.5 hours after xylose administration was greatest for AMP and least for SPC (Table 5). Increases were statistically greater for AMP than for SPC ($p<.01$) or for SPP and SPE ($p<.01$). Regression curves were generated from the xylose absorption values (Figure 4). Various parameters of these curves are reported in tables 5 and 6. Peak plasma xylose concentrations were not statistically different among diets, but tended to be greatest for AMP and least for SPC with SPP and SPE being intermediate. The xylose absorption measurements indicate that when soybean protein concentrate is included in milk replacers for preruminant calves small intestinal absorption is reduced. They also indicate that supplemental putrescine and ethylamine can partially prevent this reduction in absorption. The slopes of the regression curves (B_1) were different among diets ($p<.07$). The change in plasma xylose concentration per unit time was greater for AMP than for SPC ($p<.05$) or for SPP and SPE ($p<.10$). The slope for SPC did not significantly differ from SPP and SPE, but at day 19 of age

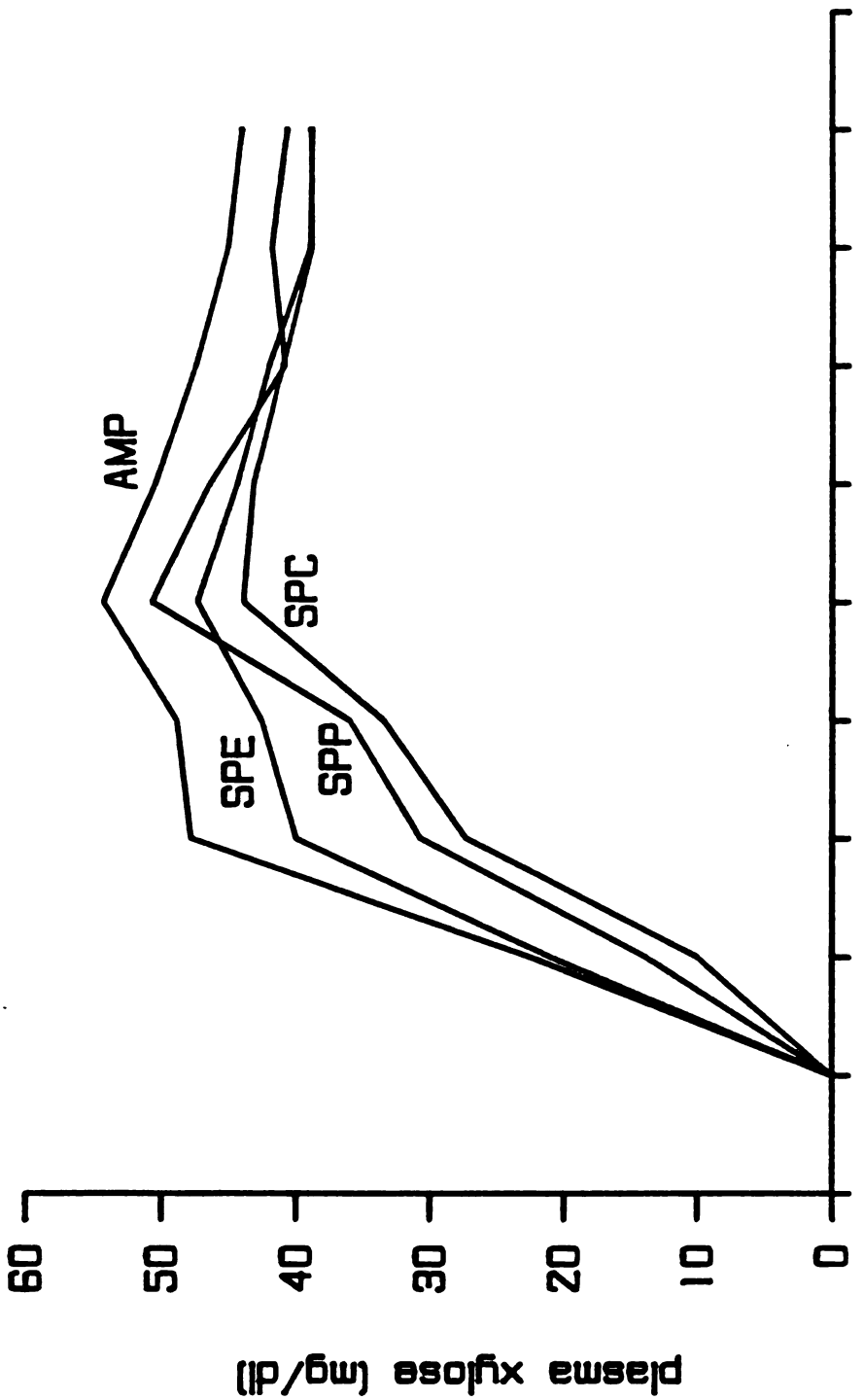


Figure 3. Xylose absorption curves of calves fed four different diets.

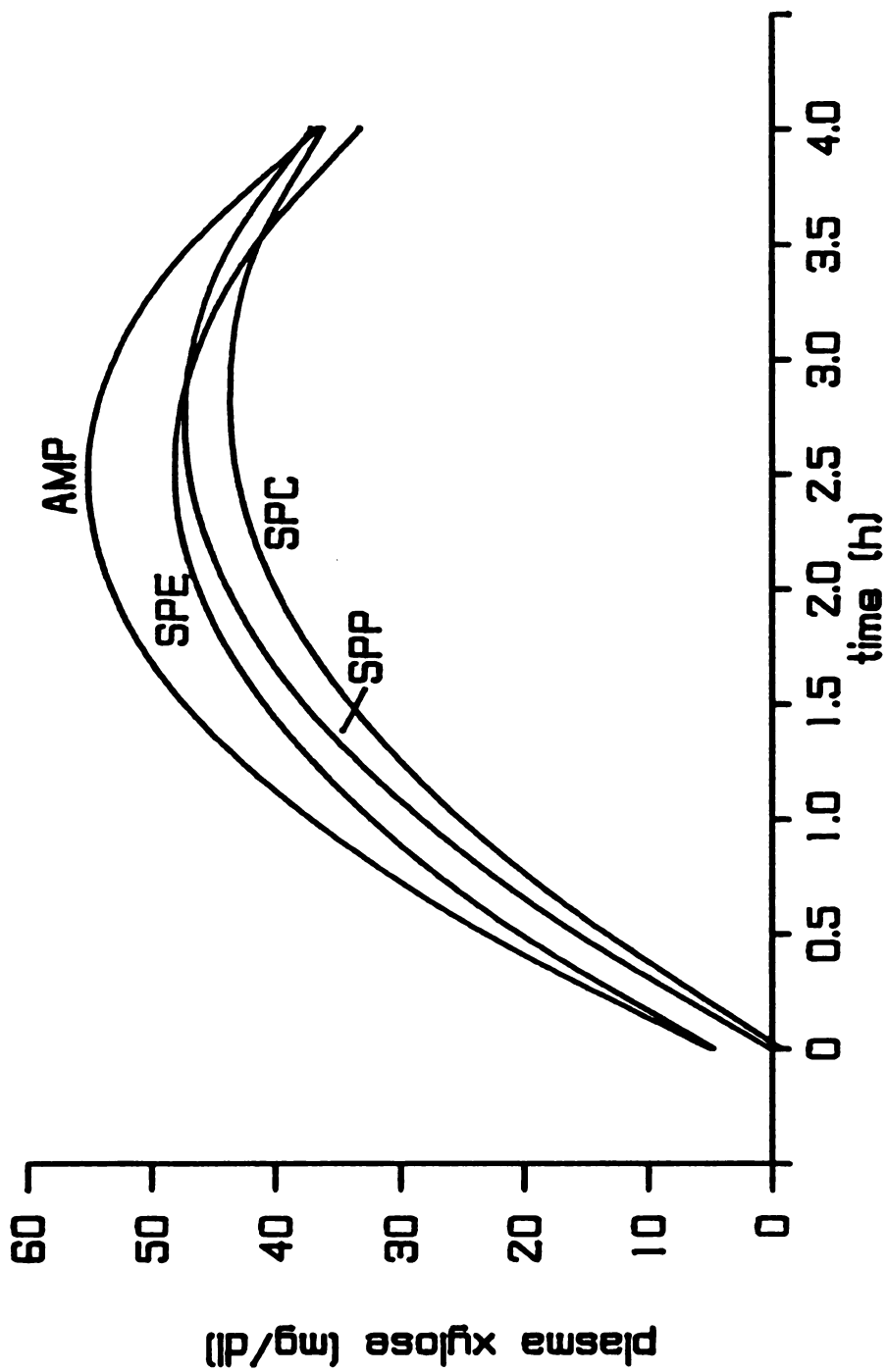


Figure 4. Regression curves generated from the xylose absorption curves of calves fed four different diets.

Table 5. Xylose absorption curve parameters for calves fed four diets.

	Diet			
	AMP	SPC	SPP	SPE
Plasma xylose 1.5 h after administration, mg/dl	48.9[a]	33.5[b]	36.1[b]	42.7[b]
SEM	0.8	0.9	0.9	0.8
Maximum plasma xylose, mg/dl	55.7	44.4	48.3	49.9
SEM	3.5	4.2	4.2	3.5
Slope of curve[c]	40.5[d]	31.8[e]	34.6[f]	34.5[f]
SEM	3.3	4.0	4.0	3.3
Curvature of curve[g]	-8.16[h]	-5.62[i]	-6.34[j]	-6.85[j]
SEM	0.9	1.1	1.1	0.9
Time of maximum plasma xylose, h	2.52	2.82	2.84	2.69
SEM	0.18	0.22	0.22	0.18

a>b ($p<.01$).

[c]Slope=B_1 of regression equation for curve; different among diets ($p<.07$).

d>e ($p<.05$).

d>f ($p<.10$).

[g]Curvature=B_2 of regression equation for curve; different among diets ($p<.02$).

h<i ($p<.01$).

h<j ($p<.02$).

Table 6. Slopes and curvatures of xylose absorption curves of calves fed four diets to three ages.

	Age	Diet				Day	
	(days)	AMP	SPC	SPP	SPE	mean	SEM
Slope[a]	5	36.1	39.3	33.7	36.7	36.5	3.1
	12	41.5	32.5	29.8	32.1	34.0	3.1
	19	43.8	23.4	40.2	34.7	35.5	3.1
Curvature[b]	5	-6.84	-6.51	-6.58	-7.66	-6.90	0.9
	12	-8.52	-6.00	-5.65	-5.86	-6.51	0.9
	19	-9.11	-4.36	-6.78	-7.02	-6.82	0.9

[a]Slope=B_1 of regression equation for curve.

[b]Curvature=B_2 of regression equation for curve.

SPC was associated with a smaller slope than the other diets (Table 6). This trend is not evident at days 5 and 12 of age. Similiar differences are found with the curvatures (B_2) of the xylose regression curves. The absolute values of the curvatures which are a reflection of the slopes and peaks of the curves were greater for AMP than for SPC ($p<.01$) or for SPP and SPE ($p<.02$). SPC curvatures were not significantly different from SPP and SPE, but tended to be least on day 19 of age (Table 6). These xylose absorption measurements indicate that calves which are fed milk replacers containing soybean protein concentrate for prolonged times (up to 19 days of age) absorb xylose at a slower rate than those fed these diets for only 3 days. These absorption observations tend to agree with those of Silva et al. (1986b) who observed that calves fed soybean protein concentrate absorbed 16% less xylose than calves fed all-milk protein milk replacer.

One could argue that the reduced xylose absortion in calves fed SPC was due to greater passage of digesta through the small intestine. However, mean times required to reach maximum plasma xylose concentrations after xylose administration was 2.7 hours for the 4 diets and were not different across diets (Table 5) suggesting that this variable (which may be indicative of flow of digesta through the gastrointestinal tract) did not influence the differences observed in xylose absorption.

Mucosal morphology:

Villus length and crypt depth measurements (Tables 7 and

8) were not reproducible. Two measurements were made in each intestinal section and variable results were obtained. Villus length results tended to be more reproducible than crypt depth in that the site difference remained significant between the two measurements ($p<.07$ and $p<.10$). Villi were longer in the proximal jejunum than in the distal jejunum of calves fed AMP, SPP, or SPE, but tended to be shorter in the proximal jejunum than in the distal jejunum of calves fed SPC. Although no significant diet differences were observed, AMP was associated with longer villi for both set of measurments when sites were combined. Crypt length was highly variable between the two sets of measurements. These results are consistent with those of Kilshaw and Slade (1982) and Seegraber and Morrill (1982 and 1986) who observed villus atrophy when heated ethanol-extracted soybean protein or heated soybean flour were fed to preruminant calves. However, these authors also reported crypt elongation in their calves which was not observed in calves in this experiment. Possibly the greater variation in size and shape of villi and crypts occurred within calves than among diets as was reported by Silva et al. (1986b). No effect of age on villus length or crypt depth was noted in this experiment which was contradictory to the of Moon and Joel (1975) which demonsrated that villus length decreased and crypt depth increased with age in calves.

Mucosal cytology:

The mitotic index of small intestinal mucosa (Table 9)

Table 7. Villus length and crypt depth in two sites of the small intestine of calves fed four diets.

		Diet				Site	
	Site[c]	AMP	SPC	SPP	SPE	mean	SEM
Villus length, #1[a], um	1	817	618	814	743	748[d]	23
	2	719	650	613	633	654[e]	23
Treatment mean		768	634	713	688		
SEM		28	40	33	33		
Villus length, #2[b], um	1	682	498	620	526	581[f]	24
	2	478	523	393	490	471[g]	24
Treatment mean		580	510	506	508		
SEM		31	42	34	34		
Crypt depth, #1[a], um	1	356	359	400	364	370	10
	2	368	362	378	328	360	10
Treatment mean		362	360	389	346		
SEM		12	18	14	14		
Crypt depth, #2[b], um	1	457	475	472	424	457	15
	2	400	436	437	406	419	15
Treatment mean		428	455	454	415		
SEM		19	25	20	20		

[a]#1=First measurement-August, 1986.

[b]#2=Second measurement-July, 1987.

[c]1=Proximal jejunum; 2=distal jejunum.

d>e ($p<.07$).

f>g ($p<.10$).

Table 8. Villus length and crypt depth in the small intestine of calves fed four diets to three ages.

	Age	Diet				Day	
	(days)	AMP	SPC	SPP	SPE	mean	SEM
Villus length, #1[a], um	7	795	593	793	709	722	28
	14	746	622	611	684	655	28
	21	763	688	736	672	715	28
Villus length, #2[b], um	7	614	414	615	488	533	31
	14	575	543	393	480	498	29
	21	552	574	511	556	548	29
Crypt depth, #1[a], um	7	388	355	419	364	381	12
	14	338	359	380	311	347	12
	21	361	367	370	364	365	12
Crypt depth, #2[b], um	7	415	463	468	448	448	19
	14	469	446	453	418	447	18
	21	401	458	441	380	420	18

[a]#1=first measurement-August, 1986.

[b]#2=second measurement-July, 1987.

was significantly influenced by diet ($p<.008$). Mitotic index is a measurement of intestinal epithelial cell proliferation and was less for SPC than for AMP ($p<.02$) or for SPP and SPE ($p<.01$) suggesting that soybean protein concentrate reduces proliferation rate of intestinal epithelium and that putrescine or ethylamine when fed with soybean protein stimulates proliferation. Diets SPP and SPE resulted in slightly greater mitotic indices than AMP ($p<.10$). No differences existed between epithelial cell proliferation of the proximal and distal jejunum nor between days.

Mucosal metabolites:

Mucosal protein concentration (Tables 9, 10, 11, and 12) was not different between diets (Table 10). A significant difference existed between Sites 1 and 2 of the intestine ($p<.01$). However, differences in protein were not consistent across all diets, so a significant diet X site interaction occurred ($p<.009$). Mucosal protein concentration was not different across diets in the proximal jejunum, but within the distal jejunum calves fed SPC had mucosal protein concentrations less than calves fed AMP ($p<.01$) or SPP and SPE ($p<.10$). Calves fed AMP had greater mucosal protein concentrations in the distal jejunum than those fed SPP and SPE ($p<.01$). This differences in protein concentrations indicate that soybean protein concentrate reduces mucosal protein concentration especially in the distal sections of the small intestine. Futhermore, addition of putrescine or ethylamine to the soybean diet partially negates the

Table 9. Small intestinal mucosal protein, RNA, and DNA concentrations and their ratios, and mitotic indices in calves fed four diets.

		Diet			
		AMP	SPC	SPP	SPE
Mitotic index, %[a]		6.30[b]	2.96[c]	7.33[d]	8.11[d]
	SEM	0.26	0.39	0.30	0.30
Protein, ug/mg wet mucosa		45.73	40.74	44.13	43.85
	SEM	1.31	2.06	1.62	1.46
RNA, ug/mg wet mucosa		6.61	7.27	7.17	7.15
	SEM	0.21	0.34	0.27	0.24
DNA, ug/mg wet mucosa		10.76	10.13	10.00	10.35
	SEM	0.26	0.41	0.32	0.29
Protein:DNA		4.41	4.15	4.59	4.34
	SEM	0.15	0.23	0.18	0.17
RNA:DNA		0.63	0.74	0.73	0.70
	SEM	0.02	0.04	0.03	0.03
Protein:RNA		7.01	5.56	6.35	6.21
	SEM	0.14	0.22	0.17	0.16

[a]Different among diets ($p<.008$).

b>c ($p<.02$).

c<d ($p<.01$).

b<d ($p<.10$).

Table 10. Mucosal protein, RNA, and DNA concentrations and their ratios in two sites of the small intestine of calves fed four diets.

		Diet				Site	
	Site[a]	AMP	SPC	SPP	SPE	mean	SEM
Protein, ug/mg wet mucosa[b]	1	44.29	43.50	46.95	45.43	45.04	1.08
	2	47.16[c]	37.98[d]	41.31[e]	42.27[e]	42.18	1.10
RNA, ug/mg wet mucosa	1	6.18	7.00	7.28	7.16	6.91	0.18
	2	7.03	7.53	7.06	7.15	7.19	0.18
DNA, ug/mg wet mucosa	1	9.83	8.83	8.57	9.22	9.11[f]	0.21
	2	11.70	11.42	11.43	11.49	11.51[g]	0.22
Protein:DNA	1	4.53	5.02	5.55	4.96	5.02[h]	0.12
	2	4.29	3.28	3.63	3.72	3.73[i]	0.12
RNA:DNA	1	0.64	0.82	0.85	0.78	0.77[j]	0.02
	2	0.62	0.67	0.62	0.63	0.63[k]	0.02
Protein:RNA	1	7.23	6.27	6.81	6.44	6.69[l]	0.12
	2	6.80	4.84	5.89	5.98	5.87[m]	0.12

[a] 1=proximal jejunum; 2=distal jejunum

[b] Different between sites ($p<.01$); Diet X site interaction ($p<.009$).

c>d ($p<.01$).

d<e ($p<.10$).

c>e ($p<.01$).

f<g ($p<.002$).

h>i ($p<.004$).

j>k ($p<.04$).

l>m ($p<.04$).

Table 11. Small intestinal mucosal protein, RNA, and DNA concentrations and their ratios in calves fed four diets to three ages.

	Age	Diet				Day	
	(days)	AMP	SPC	SPP	SPE	mean	SEM
Protein, ug/mg mucosa	7	45.55	35.68	45.38	44.00	42.65	1.42
	14	46.98	42.53	44.29	42.78	44.15	1.38
	21	44.65	44.03	42.72	44.75	44.04	1.25
RNA, ug/mg mucosa[a]	7	6.71	7.70	6.70	8.02	7.28	0.23
	14	6.80	7.85	8.09	7.17	7.48	0.23
	21	6.31	6.25	6.72	6.28	6.39	0.20
DNA, ug/mg mucosa[b]	7	11.30	9.88	9.80	10.45	10.36	0.28
	14	11.23	11.63	10.70	10.20	10.94	0.27
	21	9.75	8.88	9.50	10.41	9.63	0.25
Protein:DNA[c]	7[de]	4.25	3.90	4.85	4.29	4.32	0.16
	14[d]	4.32	3.58	4.18	4.38	4.11	0.16
	21[e]	4.67	4.98	4.74	4.36	4.69	0.14
RNA:DNA	7	0.60	0.85	0.70	0.78	0.73	0.03
	14	0.61	0.68	0.76	0.72	0.69	0.03
	21	0.67	0.70	0.74	0.60	0.68	0.02
Protein:RNA[f]	7	6.90[g]	4.34[h]	6.89[i]	5.50[i]	5.91	0.15
	14	6.96[j]	5.15[k]	5.71[l]	6.01[l]	5.96	0.14
	21	7.18	7.19	6.45	7.12	6.98	0.13

[a]Different among days ($p<.02$).
[b]Different among days ($p<.03$).
[c]Different among days ($p<.099$).
[d]<e ($p<.05$).
[f]Diet X day interaction ($p<.01$).
g>h ($p<.01$); h<i (<.02); j>k ($p<.05$); j>1 ($p<.05$).

Table 12. Mucosal protein, RNA, and DNA concentrations and protein:DNA, DNA:RNA, and protein:RNA in two sites of the small intestine of calves fed four diets to three ages.

		Diet							
		AMP		SPC		SPP		SPE	
	Age (d)	Site[a] 1	2	1	2	1	2	1	2
Protein, ug/mg mucosa	7	46.5	44.6	36.9	34.5	45.7	45.1	46.6	41.4
	14	42.6	51.4	43.2	41.9	47.5	41.1	43.3	42.3
	21	43.8	45.5	50.4	37.7	47.6	37.8	46.3	43.2
RNA, ug/mg mucosa	7	6.03	7.40	6.60	8.80	6.12	7.29	8.60	7.43
	14	5.89	7.70	7.40	8.30	8.87	7.32	6.63	7.70
	21	6.63	6.00	7.00	5.50	6.87	6.57	6.24	6.32
DNA, ug/mg mucosa[b]	7	10.36	12.24	6.40	13.35	7.97	11.63	9.53	11.37
	14	9.69	12.78	10.60	12.65	9.87	11.53	8.73	11.67
	21	9.43	10.08	9.50	8.25	7.87	11.13	9.38	11.44
Protein: DNA	7	4.57	3.92	5.60	2.19	5.82	3.88	4.91	3.67
	14	4.34	4.30	4.15	3.00	4.77	3.60	5.05	3.71
	21	4.69	4.65	5.32	4.65	6.06	3.42	4.93	3.79
RNA:DNA	7	0.58	0.62	1.02	0.68	0.77	0.63	0.91	0.66
	14	0.60	0.62	0.70	0.70	0.89	0.63	0.77	0.67
	21	0.74	0.61	0.74	0.66	0.88	0.59	0.66	0.55
Protein: RNA	7	7.71	6.10	5.65	3.02	7.56	6.22	5.43	5.57
	14	7.17	6.74	5.94	4.36	5.76	5.66	6.52	5.50
	21	6.80	7.56	7.22	7.15	7.11	5.78	7.37	6.88

[a]1=proximal jejunum;2=distal jejunum.
[b]Diet x day x site interaction ($p<.07$); day x site interaction ($p<.05$).

reduction in protein concentration in the distal jejunum.

There was no significant difference in mucosal protein content among days, but at day 7 calves receiving SPC tended to have the least mucosal protein concentration (35.7 vs 44.0 to 45.6 ug protein/mg wet tissue). This effect was not as evident at days 14 and 21 indicating that amines prevented the reduced mucosal protein that occured in newborn calves at 1 week of age when fed soybean protein concentrate.

The concentrations of DNA and RNA in small intestinal mucosa (Tables 9, 10, 11, and 12) did not differ among diets ($p>.10$). The DNA concentration was greater in the distal jejunum than in the proximal jejunum ($p<.002$). The DNA and RNA concentrations were greatest on day 14 followed by day 7 and then day 21 ($p<.03$ for DNA and $p<.02$ for RNA). A significant day X site interaction existed for DNA concentration ($p<.05$) which resulted from a diet X day X site interaction ($p<.07$). Concentration of DNA was consistently greater for Site 2 than Site 1 for all days in all diets except for day 21 of SPC in which DNA concentration was slightly less for Site 2 than for Site 1. Somewhat similiar trends were observed with RNA concentrations (Tables 9, 10, 11, and 12), but these differences were not statistically significant. Dembinski et al. (1984) also reported mucosal DNA concentration to be greater in the distal regions than in the proximal regions of the rat small intestine.

Due to the trends in protein, DNA, and RNA concentrations, ratios of protein:DNA, RNA:DNA, and protein : RNA were different between sites and/or days (Tables 9, 10,

11, and 12). If protein:DNA is indicative of epithelial cell size, RNA:DNA is indicative of the amount of protein synthesis machinery per cell, and protein:RNA is indicative of the efficiency of the cells to make protein (Allison et al., 1963; Robinson, 1969; Winick and Noble, 1965), then these data indicate that the proximal jejunum has larger epithelial cells ($p<.004$), more protein synthesis machinery per cell ($p<.04$), and is more efficient in synthesizing protein than the distal jejunum ($p<.04$). Furthermore, both the proximal and distal jejunum are more efficient in synthesizing protein at day 21 of age than day 7 ($p<.01$). However, a significant diet X day interaction existed for protein:RNA ($p<.01$). At days 7 and 14 of age, small intestinal epithelium of calves fed AMP had greater efficiencies of protein synthesis than those fed SPC ($p<.01$ for day 7 and $p<.05$ for day 14). This difference in efficiency disappeared by day 21 of age. Efficiency of protein synthesis in the small intestine at day 7 was also less for calves fed SPC than for those fed SPP and SPE ($p<.02$). These data indicate that efficiency of small intestinal epithelial cell protein synthesis is less in calves fed milk replacers containing soybean protein concentrate than in calves fed an all-milk protein milk replacer during the first 2 weeks of life. Furthermore, putrescine and ethylamine only partially prevents the reduction in protein synthesis efficiency because at 14 days of age diet AMP was associated with greater efficiencies than

were diets SPP and SPE ($p<.05$). A slight difference existed in cell size between calves at 7, 14, or 21 days of age ($p<.099$). Small intestinal epithelium of calves when 21 days of age consisted of slightly larger cells than calves at 7 and 14 days of age ($p<.05$).

Polyamine concentrations were not able to be calculated because the tissue sample used for polyamine determination was not weighed. Therefore only ratios of polyamines are valid. Putrescine:spermidine in small intestinal mucosa (Tables 13 and 14) was greatest for calves fed SPP ($p<.05$). This large difference may be due to insufficient washing of the tissue from the SPP calves resulting in contamination of tissue with ingesta. A significant diet X day interaction existed ($p<.0001$). Putrescine:spermidine was greatest on day 21 for calves fed SPP ($p<.01$).

Spermidine:spermine in intestinal mucosa (Tables 13 and 14) is an index of cellular proliferation (Hosomi et al., 1984) and this ratio tended to be greatest for SPP. If the tissue was sufficiently washed before homogenization to remove ingesta, then a greater spermidine:spermine ratio may indicate that the small intestine absorbed the dietary putrescine and converted it to spermidine. Adenosylmethionine decarboxylase, the enzyme which is rate limiting in conversion of putrescine to spermidine, is stimulated in vitro by putrescine (Tabor and Tabor, 1984) and in vivo by extracellular putrescine (Mamont and Danzin, 1981) which could explain greater spermidine:spermine in the mucosa from calves fed SPP. If the tissue was not sufficiently

Table 13.Ratios of polyamines in the small intestinal mucosa of calves fed four diets to three ages.

	Age	Diet				Day	
	(days)	AMP	SPC	SPP	SPE	mean	SEM
Put:Spd[ad]	7	1.36	2.03	3.19[b]	2.88	2.36	0.29
	14	1.36	0.45	3.34[b]	1.28	1.61	0.29
	21	1.78	1.95	8.71[c]	0.93	3.34	0.29
treatment	mean	1.50	1.48	5.08	1.70		
	SEM	0.29	0.41	0.33	0.33		
Spd:Spm[d]	7	0.38	0.47	4.34	0.35	1.39	0.53
	14	0.44	0.38	0.50	0.48	0.45	0.53
	21	0.34	0.28	0.26	0.24	0.28	0.53
treatment	mean	0.38	0.38	1.70	0.35		
	SEM	1.57	2.23	1.82	1.82		
Put:Spm[d]	7	0.51	0.88	15.82	0.96	4.54	1.95
	14	0.60	0.15	1.78	0.62	0.79	1.95
	21	0.51	0.50	2.20	0.22	0.86	1.95
treatment	mean	0.54	0.51	6.60	0.60		
	SEM	1.95	2.76	2.25	2.25		

[a]Different among diets ($p<.001$).

[a]SPP vs AMP, SPC, SPE ($p<.05$).

[a]Diet X day interaction ($p<.0001$).

b<c ($p<.01$).

[d]Put=putrescine; Spd=spermidine; Spm=spermine.

Table 14. Ratios of mucosal polyamines in two sites of the small intestine of calves fed four diets.

		Diet				Site	
	Site[a]	AMP	SPC	SPP	SPE	mean	SEM
Put:Spd[b]	1	1.66	1.99	5.53	2.08	2.82	0.24
	2	1.34	0.96	4.63	1.32	2.06	0.24
Spd:Spm[b]	1	0.33	0.32	2.83	0.30	0.95	0.43
	2	0.43	0.43	0.57	0.41	0.46	0.43
Put:Spm[b]	1	0.52	0.57	10.91	0.63	3.16	1.59
	2	0.56	0.45	2.29	0.57	0.97	1.59

[a]1=proximal jejunum; 2=distal jejunum.

[b]Put=putrescine; Spd=spermidine; Spm=spermine.

washed, then spermidine could have contaminated the calves' tissue resulting in elevated spermidine:spermine. Tabata and Johnson (1986a) suggest that polyamines in gut contents may originate from bacteria and this could have also occurred with the calves. Analysis of AMP and SPC (by the polyamine determination procedure in the appendix) indicated that putrescine and spermine were undetectable, but that concentrations of spermidine in AMP and SPC were 0.357 and 23.853 nanomoles per gram of air-dried milk replacer, respectively. However, the presence of different concentrations of spermidine in these 2 diets did not appear to have affected ratios of polyamines in intestinal mucosa.

No significant differences among diets, days, or sites existed for putrescine:spermine in the mucosa ($p>.10$; Tables 13 and 14). When Seidel et al. (1985) infused amines into the rat ileum they observed no changes in tissue concentration of polyamines, but speculated that there may have been differences in the extracellular and intracellular compartments in mucosal tissue. These individual compartments should be examined in future experiments.

Mucosal enzymes:

A significant treatment X site interaction existed for specific activity (activity expressed on a per mg tissue protein basis) of mucosal ornithine decarboxylase in mucosal tissue of the calves ($p<.05$; Table15). Specific activity in Site 1 of calves fed SPP was less than one-half that for calves fed AMP, SPC, and SPE. The difference in activity was

Table 15. Specific and total activities of mucosal ornithine decarboxylase (ODC), lactase, and dipeptidase in two sites of the small intestine of calves fed different diets.

		Diet				Site	
	Site[a]	AMP	SPC	SPP	SPE	mean	SEM
Specific activity of ODC[b]	1	117.9	119.9	52.6	170.6	115.2	20.1
	2	107.7	48.4	58.8	66.6	70.4	20.1
treatment mean		112.8	84.1	55.7	118.6		
SEM		24.3	34.4	29.8	28.0		
Total activity of ODC[c]	1	4.2	2.5	1.9	6.9	3.9	1.1
	2	3.3	0.9	2.7	2.9	2.5	1.1
treatment mean		3.7	1.7	2.3	4.9		
SEM		0.9	1.5	1.3	1.1		
Specific activity of lactase[d]	1	181.7	411.7	294.2	315.6	300.8	24.7
	2	187.5	81.7	47.8	135.6	113.1	24.3
treatment mean		184.6	246.7	171.0	225.6		
SEM		29.8	42.1	35.4	34.4		
Total activity of lactase[e]	1	7.8	12.0	9.3	14.8	11.0	0.9
	2	7.7	3.9	1.6	6.8	5.0	0.9
treatment mean		7.7	8.0	5.5	10.8		
SEM		1.5	2.4	1.2	1.7		
Specific activity of dipeptidase[f]	1	158.9	196.8	149.9	161.7	166.8[h]	7.5
	2	149.5	164.8	106.6	157.7	144.7[i]	7.4
treatment mean		154.2	180.8	128.3	159.7		
SEM		9.0	12.8	10.7	10.4		
Total activity of dipeptidase[g]	1	8.1	7.7	8.0	7.4	7.8[j]	0.5
	2	7.0	5.8	4.3	7.2	6.1[k]	0.5
treatment mean		7.5	6.8	6.2	7.3		
SEM		0.6	0.9	0.8	0.6		

[a] 1=proximal jejunum; 2=distal jejunum.
[b] pmoles CO_2 released/h/mg protein; Diet x site interaction ($p<.05$).
[c] pmoles CO_2 released/h/mg wet mucosa.
[d] nmoles lactose hydrolyzed/h/mg protein.
[e] nmoles lactose hydrolyzed/h/mg wet mucosa; Diet x site interaction ($p<.08$).
[f] nmoles phenylalanylglycine hydrolyzed/min/mg protein; Different among diets ($p<.095$).
[g] nmoles phenylalanylglycine hydrolyzed/min/mg wet mucosa.
h>i ($p<.07$); j>k ($p<.03$).

Table 16. Specific and total activities of small intestinal mucosal ornithine decarboxylase (ODC), lactase, and dipeptidase in calves fed four diets to three ages.

	Age	Diet				Day	
	(days)	AMP	SPC	SPP	SPE	mean	SEM
Specific activity of ODC[a]	7	110.6	121.5	53.3	200.1	121.4	24.9
	14	120.7	32.8	72.5	75.5	75.4	24.3
	21	107.0	98.2	41.3	80.2	81.7	24.9
Total activity of ODC[b]	7	3.7	6.7	2.1	8.8	4.6	1.5
	14	2.7	0.5	3.1	3.2	1.4	1.4
	21	4.6	4.5	1.9	4.1	3.4	1.3
Specific activity of lactase[c]	7	228.8	205.0	281.3	265.0	245.0	30.4
	14	146.3	292.5	161.7	230.0	207.6	29.8
	21	178.8	242.5	70.0	181.7	168.2	29.8
Total activity of lactase[d]	7	9.1	4.8	9.3	12.3	8.8	1.2
	14	6.2	7.7	4.1	10.0	7.0	1.1
	21	7.9	11.5	3.0	10.1	8.1	1.0
Specific activity of dipeptidase[e]	7	132.0	139.3[g]	150.5	145.2	141.8	9.2
	14	169.3	252.3[h]	126.2	150.5	174.6	9.0
	21	161.2	150.7[i]	108.0	183.5	150.9	9.0
Total activity of dipeptidase[f]	7	6.5	2.9	7.6	6.4	5.9	0.6
	14	8.8	10.6	6.2	6.5	8.0	0.6
	21	7.3	6.7	4.7	9.0	7.0	0.5

[a] pmoles CO_2 released/h/mg protein.
[b] pmoles CO_2 released/h/mg wet mucosa.
[c] nmoles lactose hydrolyzed/h/mg protein.
[d] nmoles lactose hydrolyzed/h/mg wet mucosa.
[e] nmoles phenylalanylglycine hydrolyzed/min/mg protein; Diet X day interaction ($p<.02$).
[f] nmoles phenylalanylglycine hydrolyzed/min/mg wet mucosa.
g<h ($p<.02$); h>i ($p<.05$).

not statistically significant, but the trend suggests that putrescine inhibits ornithine decarboxylase activity. Pegg et al. (1978) reported that high concentrations of liver putrescine in vitro inhibited liver ornithine decarboxylase activity. These results are contradictory to rat studies which demonstrated that when putrescine or ethylamine was ileally infused at rates equal to 1 umole/hour for 66 hours ornithine decarboxylase activity was stimulated. Such contradictory results may be due to different routes of amine administration or to different amounts of amines administered. Janne et al. (1978) reported that high concentrations of rat liver putrescine in vitro were associated with high activities of liver ornithine decarboxylase.

Specific activity of ornithine decarboxylase in Site 2 of calves fed SPC was about one-half that in calves fed AMP suggesting that soybean protein concentrate may in some way inhibit specific activity of mucosal ornithine decarboxylase in distal regions of the small intestine.

Total activities (activities expressed on a per mg wet tissue basis) of ornithine decarboxylase did not significantly differ among diets, sites, or days ($p>.10$), but the diet and site trends were similiar to those of the specific activities (Tables 15 and 16).

A significant diet X site interaction existed for the specific activities of brush border lactase ($p<.08$;Table 15). Activities were almost identical for Site 1 and Site 2 of

calves fed AMP, but were much less in Site 2 than Site 1 of calves fed SPC, SPP, and SPE. Similiar differences were observed with total lactase activities with Site 2 averaging only 11.4% of Site 1 for the three soybean diets (Table 15). These differences in lactase activity indicate that soybean protein concentrate tends to reduce lactase in the distal jejunum, but not in the proximal jejunum. Site trends were similiar to those of specific activity of ornithine decarboxylase activity in calves fed AMP, SPC, and SPE suggesting that mucosal ornithine decarboxylase may be involved in the development of brush border enzymes. Ornithine decarboxylase has been shown to be localized in the differentiating villus cells (Baylin et al., 1978) and may play roles in enterocyte differentiation (Sepulveda et al., 1982). This would include development of enzymes and transport proteins (Dahlquist, 1967; King et al., 1983: Pearse and Riecken, 1967: Trier and Madara, 1981; Yamada et al., 1981). The results of this experiment would suggest that the presence of polyamines (either from the action of ornithine decarboxylase or from dietary factors) are associated with the development of lactase since calves fed putrescine had less ornithine decarboxylase, but similiar amounts of lactase in site 1 when compared to calves of other diets. Treatment X site differences in lactase also tend to parallel changes in mucosal protein concentrations and protein:DNA and RNA:DNA ratios. All of these results together suggest that soybean protein reduces lactase in the distal jejunum by reducing villus cell size, protein synthesis

machinery per villus cell, and subsequent protein synthesis. Ornithine decarboxylase may well be a mediator of these processes because this enzyme regulates the concentration of polyamines which have been reported to enhance protein synthesis in vitro (Pingoud et al., 1984). Putrescine or ethylamine added to soybean protein concentrate tended to increase mucosal protein concentration over that of soybean protein alone, but did not enhance lactase. This increase in protein, but no change in lactase may indicate that synthesis of other proteins occurs before synthesis of lactase.

Lactase activity tended to decrease with age (Table 16). The decrease in lactase with age is consistent with studies by Huber et al. (1961) in which lactase activity in calf intestinal tissue decreased during the first few weeks of age in calves.

Specific activity of cytosolic dipeptidase (Tables 15 and 16) differed among diets ($p<.095$) and was greatest in calves fed SPC. Dipeptidase was greater on day 14 than on day 7 of age ($p<.02$), but a significant diet X day interaction occurred ($p<.02$). Dipeptidase was greater on day 14 than on day 7 or 21 for calves fed SPC ($p<.02$ and $p<.05$). This trend was also apparent for calves fed AMP, but not for calves fed SPP or SPE. Calves fed SPC had extemely high dipeptidase activity at day 14 of age as compared to days 7 or 21. This high activity in mucosa from calves fed SPC is difficult to explain, but may be related to the rate of epithelial cell proliferation. The mitotic index was least

in calves fed SPC. Low rates of crypt cell proliferation would allow villus cells to migrate slower and give them more time to differentiate. Transport of peptides in rat small intestine is initiated when cells are 30 to 35 hours of age (Cheeseman, 1986). If cells remain on villi longer, they would have more time to synthesize proteins (transport proteins and enzymes). This could explain the greater peptide hydrolase in calves fed SPC.

Specific and total activities of dipeptidase were greater in the proximal jejunum than in the distal jejunum ($p<.07$ and $p<.03$). Such results are inconsistent with those of adult sheep studies conducted by Richardson and Jouan (1986) in which dipeptidase increased throughout the jejunum and was maximum in the mid-ileum. Apparently, there are species differences or there are changes in the distribution of enzyme through the intestine as calves grow older.

Experiment 2

General observations:

In this experiment feeding the piglets with an esophageal tube was very successful. Two piglets died as a result of improper placement of esophegeal tubes, but were replaced with two other piglets. This method of feeding proved to be a convenient and successful way to feed specific quantities of liquid diets to piglets at discrete time intervals.

Three piglets had diarrhea during the experiment. One piglet died and two others were sacrificed. All three

piglets were deleted from statistical analyses. All other piglets were in excellent health throughout the experimental period. Average daily gain for all piglets was 40.5 g and was not different among diets (Table 17). Gain tended to be greatest during the second week of age for pigs fed AMP, SPI, and SPP. This is contradictory to results of Jones et al. (1977) which indicated that pigs were not able to efficiently utilize soybean protein before 14 to 18 days of age. However, Schneider and Sarrett (1969) demonstrated that soy protein isolates were superior to soybean flour diets and had 83% of the growth-promoting activity of milk protein when fed to newborn pigs. Pigs in Schneider and Sarrett's study fed soybean protein isolates gained an average of 57.1 g per day during the last 2 weeks of life (slightly greater than that observed in experiment 2). Since the protein in the soy diets used in experiment 2 consisted of only 20% soy isolate the weight gains were expected to be somewhat similar among the diet groups.

Because of changes in hematological variables in the calf experiment (experiment 1), blood samples were obtained from 22 piglets (from 6 piglets in each of diets 1, 2, and 4 and from 4 piglets in diet 3) prior to the xylose absorption tests. Samples were submitted to the Michigan State University Veterinary Clinical Center Pathology Laboratory. Results of the analyses are shown in table 18. No statistically significant differences existed among diets for packed cell volume, red blood cell and hemoglobin concentrations, or mean red cell volumes (MCV), but these

Table 17.Body weight gains of pigs fed four diets to two ages.

	Age	Diet[a]				Weekly	
	(weeks)	AMP	SPI	SPP	SPE	mean	SEM
Average daily gain, g	1	36.0	38.0	40.0	41.4	38.9	3.6
	2	43.1	40.9	45.2	38.9	42.0	3.1
Treatment mean		39.6	39.5	42.6	40.2		
SEM		4.3	4.5	5.3	5.0		

[a]AMP=all-milk protein milk replacer.

SPI=AMP with 20% of the protein replaced with soy protein isolate.

SPP=SPI plus putrescine (25g/kg dry diet).

SPE=SPI plus ethylamine (25g/kg dry diet).

There were 12 pigs/dietary treatment.

Table 18. Packed cell volume and red blood cell and hemoglobin concentrations of blood from pigs fed different diets.

	Diet			
	AMP	SPI	SPP	SPE
Packed cell volume, %	31.6	30.5	31.6	27.1
SEM	1.4	1.4	1.7	1.4
Red blood cell, $x10^6$/ul	5.19	5.27	5.37	4.83
SEM	0.24	0.24	0.29	0.24
Hemoglobin, g/dl	10.0	9.7	10.0	8.4
SEM	0.4	0.4	0.5	0.4
MCV[a], u	60.0	57.0	58.5	55.3
SEM	1.2	1.2	1.4	1.2

[a]MCV=mean red cell volume.

parameters were least for blood from piglets fed SPE. Those trends agree with results obtained in calves fed ethylamine (experiment 1) and further emphasizes that the effect of dietary amines on hemoglobin synthesis should be further investigated.

Absorption measurements:

Xylose absorption regression curves for piglets fed diets AMP, SPI, SPP, and SPE are shown in figures 5, 6, 7, and 8. Zero hour plasma xylose values (mean zero hour concentrations were 22.0 mg/dl) were subtracted from all other values so that all curves intercepted the origin. Maximum values, times of maximum values, and slopes (B_1) and curvatures (B_2) of the regression equations were not statistically significantly different among diets (Table19). Peak concentrations tended to increase from day 5 to 12 with AMP, SPP, and SPE, but did not change with SPI suggesting that soybean protein reduces the increase in small intestinal absorption that occurs in piglets during the first two weeks of life. Furthermore, putrescine or ethylamine prevented this reduction in absorption. Possibly prolonged administration (up to 2 weeks) of soybean protein isolates to young piglets results in reduced small intestinal absorption as was demonstrated with soybean protein concentrate in the calves of experiment 1. The curvatures of curves for piglets fed SPI were greatest at day 5, but least at day 12 of age which is consistent with the suggestion that prolonged administration of soybean protein isolates reduce small

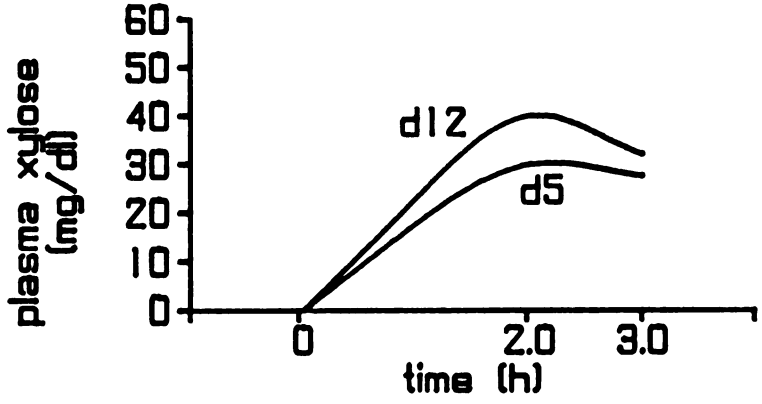


Figure 5. Regression curves generated from the xylose absorption curves of pigs fed AMP to day 5 and day 12 of age.

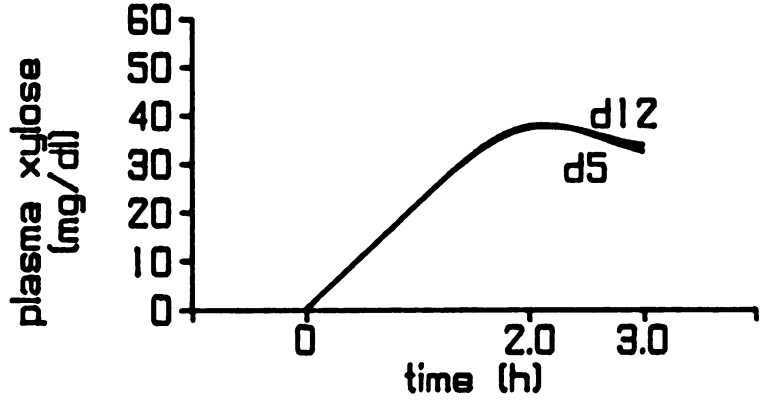


Figure 6. Regression curves generated from the xylose absorption curves of pigs fed SPI to day 5 and day 12 of age.

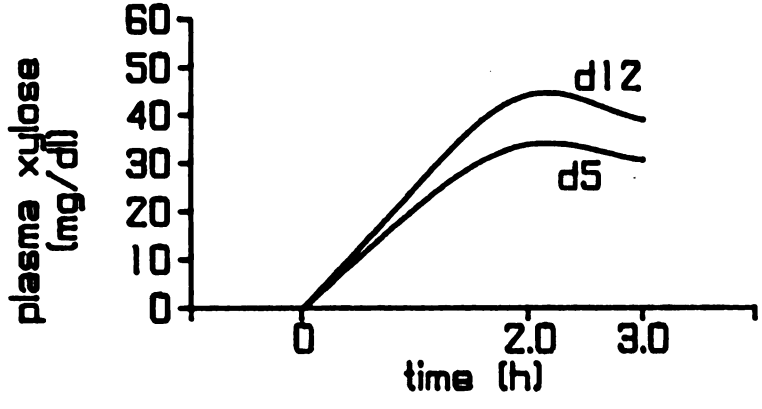


Figure 7. Regression curves generated from the xylose absorption curves of pigs fed SPP to day 5 and day 12 of age.

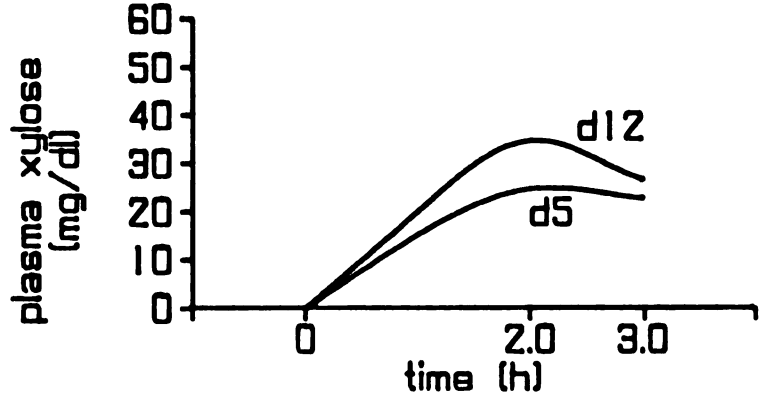


Figure 8. Regression curves generated from the xylose absorption curves of pigs fed SPE to day 5 and day 12 of age.

Table 19. Xylose absorption curve parameters for pigs fed four diets to two ages.

	Age	Diet				Day	
	(days)	AMP	SPI	SPP	SPE	mean	SEM
Maximum plasma xylose, mg/dl	5	30.7	38.3	34.5	26.0	32.2	1.8
	12	40.6	38.4	44.9	35.2	39.3	1.8
Treatment mean		35.7	38.4	39.7	30.6		
SEM		2.4	2.4	2.9	2.4		
Time of maximum plasma xylose, h	5	2.29	2.29	2.25	2.44	2.32	0.05
	12	2.08	2.25	2.21	2.09	2.15	0.05
Treatment mean		2.18	2.27	2.23	2.27		
SEM		0.07	0.07	0.08	0.07		
Slope of curve[a]	5	26.6	34.1	30.5	22.5	28.2	1.6
	12	39.3	35.2	40.8	34.2	37.1	1.6
Treatment mean		32.9	34.7	35.7	28.4		
SEM		2.1	2.1	2.6	2.1		
Curvature of curve[b]	5	-5.77	-7.65	-6.74	-4.96		
	12	-9.53	-8.13	-9.30	-8.43		
Treatment mean		-7.64	-7.88	-8.01	-6.69		
SEM		0.55	0.55	0.67	0.55		

[a]Slope=B_1 of regression equation for curve.

[b]Curvature=B_2 of regression equation for curve.

intestinal absorption.

Mucosal morphology:

Villus length and crypt depth data are presented in tables 20 and 21. Villus length and crypt depth were not different among diets. Villi were longer at day 7 than at day 14 of age ($p<.0001$) and length was different among sites ($p<.0001$); villi of the duodenum were shorter than villi of the jejunum ($p<.01$) and ileum ($p<.01$). Moon (1971) observed that villus length decreases during the first few weeks of life in pigs. This author also reported crypt depth to increase with age which may indicate that the size of the proliferative compartment for intestinal epithelial cells increases with age. In the present experiment, crypt depth tended to be greater at day 14 than at day 7 of age. Crypt depth was least in the jejunum of all piglets ($p<.01$) and was least in all piglets fed SPI suggesting that soybean protein reduces intestinal proliferation in piglets.

Mucosal cytology:

In this experiment, mitotic indices of small intestinal mucosa (Tables 20 and 21) were not influenced by diet. Mitotic index was greatest at day 7 of age and was greater in the jejunum than in the duodenum ($p<.01$) and ileum ($p<.10$). These data would indicate that proliferation of intestinal epithelium is greatest at day 7 of age and greatest in the jejunum which is inconsistent with crypt depth measurements. In addition to crypt depth, number of crypts should have been

Table 20. Villus length, crypt depth, and mitotic index in the small intestine of pigs fed four diets to two ages.

	Age	Diet				Day	
	(days)	AMP	SPI	SPP	SPE	mean	SEM
Villus length, um	7	438	421	443	416	430[a]	16
	14	245	298	197	231	242[b]	16
Treatment mean		341	359	320	324		
SEM		22	22	23	23		
Crypt depth, um	7	764	683	687	780	728	29
	14	747	703	824	785	765	30
Treatment mean		755	693	756	783		
SEM		41	44	41	41		
Mitotic index, %	7	3.2	3.0	3.2	2.9	3.1[c]	0.1
	14	2.4	2.6	2.2	2.3	2.4[d]	0.1
Treatment mean		2.8	2.8	2.7	2.6		
SEM		0.2	0.2	0.2	0.2		

a>b ($p<.0001$).

c>d ($p<.01$).

Table 21. Villus length, crypt depth, and mitotic index in three sites of the small intestine of pigs fed four diets.

		Diet				Site	
	Site[a]	AMP	SPI	SPP	SPE	mean	SEM
Villus length, um	1	252	285	186	223	237[b]	20
	2	395	386	431	384	399[c]	19
	3	377	406	342	363	372[c]	20
Crypt depth, um	1	829	787	838	962	854[e]	36
	2	633	560	629	570	598[d]	36
	3	804	732	800	815	788[e]	36
Mitotic index, %	1	2.4	2.4	2.3	2.3	2.3[f]	0.15
	2	3.1	3.6	3.2	2.7	3.2[g]	0.15
	3	2.9	2.5	2.6	2.8	2.7[h]	0.15

[a]1=duodenum; 2=jejunum; 3=ileum.

b<c ($p<.01$).

d<e ($p<.01$)

f<g ($p<.01$).

g>h ($p<.10$).

measured in this experiment. An increase in crypt number with advancing age has been observed in pigs (Moon, 1971) and may have a greater effect on proliferative rate than crypt depth. Measuring mitotic index only estimates the fraction of cells proliferating, not the absolute number of cells proliferating. An increase in crypt cell number would not be detected with mitotic indices measurements nor with crypt depth measurements. Future trials should include measurement of number of crypt cells.

Mucosal metabolites:

A significant diet X day interaction ($p<.008$) existed for small intestinal mucosal protein concentration (Table 22). At 14 days of age, concentration of mucosal protein in pigs fed SPP and SPE was less than in pigs fed AMP ($p<.01$) or SPI ($p<.10$). There was a tendency for protein concentration to increase with age in pigs fed AMP and SPI, but protein decreased in pigs fed SPP ($p<.05$) and SPE ($p<.10$). Protein concentration differed among sites ($p<.02$) and was less in the jejunum than the duodenum ($p<.05$; Table 23) which parallels the site differences observed with the crypt depth measurements.

The concentration of DNA in small intestinal mucosa (Tables 22 and 23) was greater in pigs fed AMP than in pigs fed SPP and SPE ($p<.10$), whereas the mucosal DNA concentration in pigs fed SPI was intermediate. Age had no significant effect on mucosal DNA concentration, but DNA tended to increase with age in pigs fed AMP and SPI and to

Table 22. Small intestinal mucosal protein, RNA, and DNA concentrations and their ratios in pigs fed four diets to two ages.

	Age	Diet				Day	
	(days)	AMP	SPI	SPP	SPE	mean	SEM
Protein, ug/mg wet mucosa[a]	7	51.07	50.14	54.52[e]	54.94[g]	52.67	0.75
	14	57.01[b]	52.88[c]	49.94[df]	44.71[dh]	49.38	0.75
Treatment mean		54.04	51.51	48.73	49.83		
SEM		1.03	1.10	1.03	1.10		
DNA, ug/mg wet mucosa	7	5.19	4.73	4.76	4.99	4.92	0.10
	14	5.39	5.36	4.51	4.51	4.94	0.10
Treatment mean		5.29[i]	5.05	4.63[j]	4.75[j]		
SEM		0.13	0.14	0.13	0.15		
RNA, ug/mg wet mucosa	7	5.26	5.55	5.10	5.45	5.34[k]	0.10
	14	5.31	5.14	4.46	4.70	4.90[l]	0.10
Treatment mean		5.28	5.34	4.78	5.08		
SEM		0.13	0.14	0.13	0.14		
Protein:DNA	7	9.99	10.69	11.62	11.20	10.87	0.22
	14	10.84	10.01	10.23	10.07	10.29	0.24
Treatment mean		10.41	10.35	10.93	10.63		
SEM		0.31	0.31	0.31	0.32		
RNA:DNA	7	1.03	1.18	1.11	1.13	1.12[m]	0.03
	14	1.01	0.99	1.03	0.99	1.00[n]	0.03
Treatment mean		1.02	1.09	1.07	1.06		
SEM		0.04	0.04	0.04	0.05		
Protein:RNA[o]	7	9.80	9.15	10.77	10.17	9.97	0.18
	14	10.95	10.36	9.70	10.46	10.37	0.19
Treatment mean		10.37	9.76	10.23	10.31		
SEM		0.25	0.27	0.25	0.28		

[a]Diet X day interaction ($p<.008$).
b>c ($p<.01$).
c>d ($p<.10$).
e>f ($p<.05$).
g>h ($p<.10$)
i>j ($p<.10$).
k>l ($p<.02$).
m>n ($p<.04$).
[o]Diet X day interaction ($p<.09$).

Table 23. Mucosal protein, RNA, and DNA concentrations and their ratios in three sites of the small intestine of pigs fed four diets.

		Diet				Site	
	Site[a]	AMP	SPI	SPP	SPE	mean	SEM
Protein, ug/mg wet mucosa[b]	1	55.84	53.20	49.60	51.91	52.64[d]	0.92
	2	51.74	50.41	46.68	47.01	48.96[c]	0.92
	3	54.55	50.91	49.91	50.55	51.48	0.92
DNA, ug/mg wet mucosa	1	4.93	4.85	4.35	4.72	4.71[e]	0.12
	2	5.00	4.95	4.13	4.23	4.58[e]	0.12
	3	5.94	5.33	5.42	5.30	5.50[f]	0.12
RNA, ug/mg wet mucosa	1	5.67	5.87	5.03	5.82	5.60[g]	0.12
	2	5.18	5.02	4.80	4.64	4.91[h]	0.12
	3	5.01	5.14	4.50	4.77	4.86[h]	0.12
Protein:DNA	1	11.40	10.96	12.23	11.35	11.49[i]	0.28
	2	10.49	10.32	11.23	11.13	10.79[i]	0.28
	3	9.33	9.78	9.32	9.41	9.46[j]	0.28
RNA:DNA	1	1.16	1.22	1.21	1.27	1.21[k]	0.04
	2	1.05	1.03	1.16	1.09	1.08[k]	0.04
	3	0.86	1.00	0.84	0.83	0.88[l]	0.04
Protein:RNA	1	9.92	9.10	9.90	9.15	9.52[m]	0.23
	2	10.23	10.15	9.79	10.31	10.12[n]	0.23
	3	10.97	10.02	11.01	11.47	10.87[o]	0.23

[a]1=duodenum; 2=jejunum; 3=ileum.
[b]Different between sites ($p<.02$).
c<d ($p<.05$); e<f ($p<.01$); g>h ($p<.01$); i>j ($p<.01$); k>l ($p<.01$); m<o ($p<.01$); n<o ($p<.05$).

decrease with age in pigs fed SPP and SPE. Mucosal DNA was also greatest in the ileum ($p<.01$) for all diets. The greater DNA concentration in the distal small intestine agrees with results in calves (experiment 1) and in rats (Dembinski, 1984).

Mucosal RNA concentration (Tables 22 and 23) was not different among diets, but also tended to be greater for pigs fed AMP and SPI than for pigs fed SPP and SPE. Mucosal RNA decreased from day 7 to day 14 of age ($p<.02$) for diets SPI, SPP, and SPE. Differences between days 7 and 14 were greatest for pigs fed SPP and SPE. Conversely, concentrations of RNA in pigs fed AMP increased very slightly from day 7 to day 14 of age. Mucosal RNA was also greatest in the duodenum ($p<.01$) for all diets (Table 23) which is consistent with rat studies by Dembinski et al. (1984) in which RNA was greatest in the more proximal regions of the small intestine.

Mucosal protein:DNA ratio in the small intestine (Tables 22 and 23) was less in the ileum than in the jejunum ($p<.01$) or in the duodenum ($p<.01$) indicating that intestinal epithelial cells are larger in the duodenum and jejunum than in the ileum. This same trend was oberved with RNA:DNA (Tables 22 and 23) suggesting that cells of the ileum have less protein synthesis machinery than those of the proximal two sections of the small intestine ($p<.01$ and $p<.01$). The same differences have been observed in rats (Dembinski,1984). Amount of protein synthesis machinery decreased with age as was indicated by greater RNA:DNA at day 7 than at day 14 of age ($p<.04$). Mucosal protein: RNA (Tables 22 and 23) was

greater in the ileum than in the duodenum ($p<.01$) and the jejunum ($p<.05$) suggesting that efficiency of protein synthesis was greatest in the ileal mucosa. A significant diet X day interaction ($p<.09$) indicates a tendency for greater efficiencies of mucosal protein synthesis on day 14 than on day 7 of age for pigs fed AMP, SPI, and SPE, but lower efficiencies on day 14 of age for pigs fed SPP.

The trends in small intestinal mucosal protein, DNA, and RNA concentrations indicate that putrescine and ethylamine may be increasing rates of villus cell sloughing. Villi became shorter with age. A reduction in villus length coupled with a crypt cell production rate which remains the same or increases with age would necessarily mean a greater rate of villus cell sloughing. If putrescine or ethylamine are increasing crypt cell production rates and/or reducing villus height, then this could explain the reductions in mucosal protein, DNA, and RNA that accompanied increased age (Table 22). Indeed, villus length was least in piglets fed putrescine and ethylamine at 14 days of age (Table 20).

Mucosal protein:DNA and RNA:DNA site differences in the pigs of this experiment are consistent with those of calves in experiment 1 in that the size of intestinal epithelial cells and amount of protein synthesis machinery per cell was least in the more distal regions of the small intestine. This experiment is contradictory to the calf experiment in that calf mucosal protein:RNA was greater in the more proximal regions rather than the more distal regions of the

small intestine indicating that site differences in efficiency of mucosal protein synthesis exist among species.

Concentrations of polyamines in the small intestinal contents are presented in table 24. Putrescine tended to be greater in intestinal contents from pigs fed putrescine as would be expected, however, due to the very great variation this was not statistically significant. The contents were collected from the jejunum. Gut microbes may metabolize putrescine to spermidine and spermine. Thus any variation in distribution of microbes or in microbe populkations in the small intestine may contribute to variations in distribution of putrescine.

Spermidine and spermine in intestinal contents were not influenced by diet. Spermine in intestinal contents was greater on day 14 than on day 7 of age. This may be explained by a change in the gut microbial population to one which may metabolize luminal polyamines to spermine. Increases in spermidine and spermine concentrations over age are greater for diets containing soybean protein. Analysis of AMP and SPI (by the polyamine determination procedure described in the appendix) revealed that polyamines were undetectable in AMP and that putrescine and spermine were undetectable in SPI. However, SPI contained 24.164 nanomoles of spermidine per gram of air-dried feed. If gut microbes do metabolize spermidine and spermine, then this could explain the greater spermine in the intestinal contents of pigs fed SPI, SPP, and SPE at 14 days of age.

Concentrations of polyamines in small intestinal mucosa

Table 24. Concentration of polyamines in the small intestinal contents of pigs fed four diets to two ages.

	Age	Diet				Day	
	(days)	AMP	SPI	SPP	SPE	mean	SEM
Putrescine, nmoles/ml contents	7	34.4	32.8	868.9	79.6	253.9	25.6
	14	7.0	50.1	996.0	19.7	268.2	26.9
Treatment mean		20.7	41.4	932.4	49.6		
SEM		33.8	44.3	35.3	37.1		
Spermidine, nmoles/ml contents	7	2.5	3.2	2.6	1.4	2.4	0.7
	14	1.5	6.6	3.3	1.8	3.3	0.7
Treatment mean		2.0	4.9	3.0	1.6		
SEM		1.0	1.0	1.0	1.0		
Spermine, nmoles/ml contents	7	6.2	8.5	5.5	4.0	6.0	2.7
	14	6.9	15.1	9.8	10.3	10.5	2.3
Treatment mean		6.5	11.8	7.6	7.2		
SEM		3.6	3.6	3.3	3.4		

Table 25. Concentration and ratios of mucosal polyamines in three sites of small intestine of pigs fed four diets.

		Diet				Site	
	Site[a]	AMP	SPI	SPP	SPE	mean	SEM
Putrescine,	1	96.6	33.0	195.9	60.2	96.5	22.1
pmoles/mg	2	70.8[c]	32.2[c]	759.5[d]	34.3[c]	224.2	20.3
wet mucosa[b]	3	32.4[e]	84.6[e]	714.8[f]	39.7[e]	217.9	21.1
Treatment mean		66.6	49.9	556.7	44.7		
SEM		26.1	26.1	22.0	23.8		
Spermidine,	1	149.5	96.7	106.4	111.0	115.9	9.1
pmoles/mg	2	107.4	90.7	103.4	79.6	95.3	8.9
wet mucosa	3	126.9	87.7	116.4	92.9	106.0	9.3
Treatment mean		127.9	91.7	108.7	94.5		
SEM		9.3	11.7	11.7	9.9		
Spermine,	1	285.9	162.9	187.5	342.3	244.7	23.8
pmoles/mg	2	281.9	209.8	221.4	311.6	256.2	22.7
wet mucosa	3	345.1	184.4	216.5	364.0	277.5	23.2
Treatment mean		304.3	185.7	208.5	339.3		
SEM		23.7	28.1	31.5	25.7		
Put:Spd[g]	1	2.89	1.49	27.93	1.46	8.44	3.18
	2	2.80	2.60	64.29	1.32	17.75	3.42
	3	2.62	3.82	59.85	1.25	16.88	3.13
Treatment mean		2.77	2.64	50.69	1.34		
SEM		3.56	3.81	4.11	3.56		
Spd:Spm[g]	1	1.40	1.34	6.05	0.41	2.11	1.00
	2	0.77	2.51	0.67	0.30	0.56	1.00
	3	0.58	4.69	5.29	0.38	1.63	0.98
Treatment mean		0.89	2.92	4.29	0.37		
SEM		1.04	1.10	1.46	1.12		
Put:Spm[g]	1	0.44	0.43	47.75	0.63	12.31	7.74
	2	0.26	0.98	10.34	1.64	3.31	8.39
	3	0.18	4.12	22.93	2.45	7.42	7.34
Treatment mean		0.29	1.85	27.01	1.57		
SEM		8.78	9.23	10.06	8.21		

[a]1=duodenum; 2=jejunum; 3=ileum.
[b]Diet X site interaction ($p<.0001$).
c<d ($p<.002$).
e<f ($p<.002$).
[g]Put=putrescine; Spd=spermidine; Spm=spermine.

Table 26. Concentrations and ratios of polyamines in the small intestinal mucosa of pigs fed four diets to two ages.

	Age	Diet				Day	
	(days)	AMP	SPI	SPP	SPE	mean	SEM
Putrescine, pmoles/mg wet mucosa[a]	7	51.2	71.1	603.7	41.8	192.0	16.9
	14	82.0	28.7	509.8	47.6	167.0	17.6
Spermidine, pmoles/mg wet mucosa	7	101.2	92.0	82.9	102.5	94.7	7.9
	14	154.6	91.4	134.5	86.5	116.8	7.0
Spermine, pmoles/mg wet mucosa[b]	7	194.8	103.5	212.9	274.7	196.5[c]	19.9
	14	413.8	267.9	204.0	403.9	322.4[d]	18.2
Put:Spm[eh]	7	5.15[g]	4.91[g]	97.18[f]	2.05[g]	27.32	2.79
	14	0.39	0.37	4.19	0.63	1.39	2.52
Spd:Spm[h]	7	1.00	3.39	4.65	0.46	0.87	0.89
	14	0.81	2.70	3.81	0.29	1.99	0.75
Put:Spm[h]	7	0.18	1.18	22.07	2.87	6.57	7.00
	14	0.41	2.51	31.95	0.28	8.79	5.87

[a]Different among diets ($p<.0001$).

[b]Diet X day interaction ($p<.06$).

c<d ($p<.11$).

[e]Diet X day interaction ($p<.08$).

f>g ($p<.002$).

[h]Put=putrescine; Spd=spermidine; Spm=spermine.

are presented in Tables 25 and 26. Mucosal putrescine was greatest in the jejunum and ileum of pigs fed SPP ($p<.002$). Duodenal mucosal concentrations of putrescine among diets were not different. This greater concentration of putrescine in the jejunum and ileum suggests that the distal regions of the small intestine take up more putrescine from the luminal contents than the proximal regions. Kumagai and Johnson (1987) found that the uptake of putrescine by rat small intestinal enterocyttes was also more extensive in the distal regions of the small intestine. Hosomi et al. (1984) demonstrated in fasted rats that mucosal putrescine concentrations decreased from the duodenum to the colon. This decrease in mucosal putrescine concentration as one traverses down the intestine was noted in this experiment for pigs fed AMP, but not for pigs fed SPI suggesting that soybean protein alters the intestinal uptake of or biosynthesis of putrescine.

Mucosal spermidine tended to be greater in pigs fed AMP than for pigs fed SPI and pigs fed SPP and SPE. Spermidine was also greater on day 14 than 7 for all diets, but age changes varied among diets. Spermine concentration was not different among diets and sites, but was greatest on day 14 of age ($p<.11$) for all diets and tended to be least in pigs fed SPI. These results suggest that soybean protein reduces the concentration of mucosal spermidine and that putrescine may alter mucosal polyamine metabolism.

Mucosal putrescine:spermidine was greater in pigs fed

SPP than in pigs fed AMP, SPI, and SPE at day 7 of age ($p<.002$). Greater mucosal putrescine:spermidine ratios were also observed in calves fed SPP than in calves fed SPC, SPE, and AMP (experiment 1). This difference was not statistically significant at day 14 of age for pigs. Spermidine:spermine (an index of cellular proliferation) tended to decrease from the duodenum to the ileum in pigs fed AMP as was demonstrated in fasted rats by Hosomi et al. (1984). However, in pigs fed SPI, the ratio increased from duodenum to ileum suggesting that soybean protein altered intestinal proliferation. Mucosal putrescine:spermine tended to be greatest in pigs fed SPP as would be expected if the intestine takes up putrescine from the luminal contents.

Mucosal enzymes:

Specific and total activities of mucosal ornithine decarboxylase (Tables 27 and 28) were least in pigs fed SPP ($p<.01$ and $p<.002$ for specific and total activities, respectively). Specific activity was greater in the duodenum than in the jejunum ($p<.01$) and total activity was greater in the duodenum than in the jejunum ($p<.01$) and the ileum ($p<.05$). However, a significant diet X site interaction ($p<.02$) occurred for total ornithine decarboxylase activity and day X site interactions occurred for specific ($p<.001$) and total ($p<.002$) activities. Specific and total activities in all 3 intestinal sites were lowest for pigs fed SPP indicating that the added dietary putrescine inhibited the activity of mucosal ornithine decarboxylase. This trend was

Table 27. Specific and total activities of mucosal ornithine decarboxylase (ODC), sucrase, and dipeptidase in three sites of the small intestine of pigs fed four diets.

		Diet				Site	
	Site[a]	AMP	SPI	SPP	SPE	mean	SEM
Specific activity of ODC[v]	1	34.8	31.1	9.0	47.4	30.6	2.4
	2	22.4	25.8	5.6	27.3	20.3	2.4
	3	34.0	29.0	9.5	27.0	24.9	2.4
Treatment mean		30.4[c]	28.6[c]	8.0[b]	33.9[c]		
SEM		2.7	2.7	2.8	2.8		
Total activity of ODC[fw]	1	1.33	1.40	0.42	2.17	1.33	0.08
	2	0.96	1.06	0.24	1.00	0.82	0.08
	3	1.36	1.29	0.38	1.08	1.03	0.08
Treatment mean		1.22[e]	1.25[e]	0.35[d]	1.41[e]		
SEM		0.10	0.09	0.10	0.10		
Specific activity of sucrase[x]	1	541	438	364	362	426	25
	2	398	411	288	372	367	25
	3	426	387	404	296	378	25
Treatment mean		455	412	352	343		
SEM		28	28	30	30		
Total activity of sucrase[y]	1	25.6	20.7	17.9	19.0	20.8[g]	1.0
	2	18.0	17.6	12.1	15.8	15.9[h]	1.0
	3	18.5	18.3	15.9	13.6	16.6[i]	1.0
Treatment mean		20.7	18.9	15.3	16.1		
SEM		1.2	1.2	1.2	1.2		
Specific activity of dipeptidase[z]	1	557	484	426	450	480[j]	27
	2	932	973	713	944	891[k]	27
	3	834	695	552	742	706[l]	27
Treatment mean		774[ps]	718[s]	564[qr]	712[qs]		
SEM		31	31	32	32		
Total activity of dipeptidase[zz]	1	26.3	21.9	20.2	20.6	22.3[m]	1.2
	2	40.6	41.6	31.4	37.1	37.7[n]	1.2
	3	34.2[t]	32.1	24.5[u]	29.3[u]	30.0[o]	1.2
Treatment mean		33.7	31.9	25.4	29.0		
SEM		1.3	1.3	1.4	1.4		

[a]1=duodenum, 2=jejunum; 3=ileum.
b<c ($p<.01$); d<e ($p<.002$); [f]Diet site interaction ($p<.02$); g>h ($p<.01$); g>i ($p<.02$); j<l<k ($p<.01$); m<o<n ($p<.01$); p>q ($p<.10$); r<s ($p<.05$); t>u ($p<.10$).
[v]pmoles CO_2 released/h/mg protein.
[w]pmoles CO_2 released/h/mg wet mucosa.
[x]nmoles sucrose hydrolyzed/h/mg protein.
[y]nmoles sucrose hydrolyzed/h/mg wet mucosa.
[z]nmoles phenylalanylglycine hydrolyzed/min/mg protein.
[zz]nmoles phenylalanylglycine hydrolyzed/min/mg wet mucosa.

Table 28. Specific and total activities of mucosal ornithine decarboxylase (ODC), sucrase, and dipeptidase in three sites of the small intestine of pigs at two ages.

	Age	Site[a]			Day	
	(days)	1	2	3	mean	SEM
Specific activity of ODC[br]	7	37.3[c]	14.1[d]	21.7[d]	24.4	1.9
	14	23.9	26.4	28.0	26.1	1.9
Total activity of ODC[s]	7	1.56[f]	0.63[g]	0.92[g]	1.04	0.07
	14	1.11	1.00	1.13	1.08	0.07
Specific activity of sucrase[t]	7	409	334	344	362	20
	14	444	401	412	419	21
Total activity of sucrase[u]	7	21.0	16.1	15.9	17.7	0.8
	14	20.5	15.6	17.2	17.8	0.8
Specific activity of dipeptidase[v]	7	490	987[j]	770[l]	749	22
	14	469	794[k]	642[m]	635	23
Total activity of dipeptidase[w]	7	22.6	43.1[n]	34.1[p]	33.3	0.9
	14	21.9	32.3[o]	26.0[q]	26.7	1.0

[a] 1=duodenum; 2=jejunum; 3=ileum.
[b] Day X site interaction ($p<.001$).
[c] >d ($p<.01$).
[e] Day X site interaction ($p<.002$).
[f] >g ($p<.01$).
[h] Day X site interaction ($p<.09$).
[i] Day X site interaction ($p<.01$).
[j] >k ($p<.01$).
[l] >m ($p<.10$).
[n] >o ($p<.01$).
[p] >q ($p<.01$).
[r] pmoles CO_2 released/h/mg protein.
[s] pmoles CO_2 released/h/mg wet mucosa.
[t] nmoles sucrose hydrolyzed/h/mg protein.
[u] nmoles sucrose hydrolyzed/h/mg wet mucosa.
[v] nmoles phenylalanylglycine hydrolyzed/min/mg protein.
[w] nmoles phenylalanylglycine hydrolyzed/min/mg wet mucosa.

evident in the proximal jejunum of calves in experiment 1. However, soybean protein did not tend to reduce activities of ornithine decarboxylase as was suggested in experiment 1. This may be due to species differences or to differences in soybean proteins. Total and specific activities were also greatest in the duodenum of piglets at 7 days of age ($p<.01$), but this difference disappeared by 14 days of age.

Specific and total activities of intestinal brush border sucrase (Tables 27 and 28) were not significantly influenced by diet, but tended to be greatest in pigs fed AMP and least in pigs fed SPP and SPE. This was most evident in the duodenum. Total activity was greater in the duodenum than in the jejunum ($p<.01$) and ileum ($p<.02$) for all diets. Several authors (Alpers and Tedesco, 1975; Goda et al.,1985; Thornberg et al., 1987) suggested that the pancreatic proteases play roles in degrading brush border enzymes. The duodenum samples in the present experiment were obtained proximal to the pancreatic duct and which may help explain the greater sucrase activity in the duodenum. Sucrase activities (total and specific) were not altered by diet in the jejunum and ileum suggesting that pancreatic secretions were sufficient in those pigs fed a soy protein isolate to degrade sucrase. Pancreatic enzyme secretion was not altered in pigs fed raw soybeans in studies by Hooks et al. (1965), but in studies by Efird et al. (1982) piglets that were fed soy flour had greater pancreatic enzyme activities in intestinal contents than piglets fed milk. The conflicting results indicate that different soybean proteins produce

variable results on pancreatic enzyme secretion or activity. Greater activity of sucrase in the proximal small intestine may also be related to greater RNA:DNA in this region. More protein synthesis machinery per epithelial cell could have resulted in greater synthesis of sucrase.

Specific and total activities of cystolic dipeptidase (Tables 27 and 28) were greater in pigs fed AMP than in pigs fed SPP and SPE ($p<.10$). Specific activity was least in pigs fed SPP ($p<.05$). Activities were greatest in the jejunum ($p<.01$) of pigs of all diets. This greater activity in the jejunum is consistent with data from adult pig studies in which dipeptidase was maximum in the jejunum (Josefsson and Lindberg, 1965). Specific activity of dipeptidase decreased from day 7 to day 14 of age in the jejunum and ileum ($p<.01$ and $p<.10$ for jejunum and ileum, respectively). Similiar differences existed for total activities ($p<.01$ for both jejunum and ileum).

The trends in sucrase and dipeptidase activities in the small intestinal mucosa provide further support for the idea that putrescine and ethylamine may reduce villus length by cell sloughing. Greater sloughing of villus cells in pigs fed SPP and SPE would explain why activities of sucrase and peptidase were greater in pigs fed AMP than in pigs fed SPP and SPE. Furthermore, if putrescine or ethylamine increased crypt cell production rate, then villus cells would have even less time to mature before being sloughed off because villus length was not changed. This would result in an immature

villus cell population and subsequently lead to the synthesis of smaller quantities of sucrase and peptidase.

Experiment 3

General observations:

All eight pigs in this experiment were in excellent health throughout the entire experimental period. Feeding success was excellent and there was no evidence of diarrhea. Average daily gain was 54.4 g for all pigs and was not different between diets (Table 29). Body weight gains were greater in this experiment than in experiment 2. This was probably due to the higher crude protein resulting from the addition of soy protein isolate to both diets of this experiment. Putrescine supplementation led to a slight increase in body weight gain, but this was not statistically significant.

Blood samples were collected from all piglets prior to xylose absorption tests and analyzed for packed cell volume, red cell volume, and hemoglobin and red blood cell concentrations as was done in experiment 2. Results are shown in table 29. Diet had no effect on any hematological variable measured. Blood variables of pigs and calves fed soybean protein or SPP did not differ in experimenet 1 and 2, either, thus indicating that putrescine has no effect on these blood parameters. Red cell numbers and hemoglobin concentrations were less for pigs in experiment 3 than experiment 2. This difference could be due to the difference in age (blood variables were measured at day 14 rather than

Table 29. Body weight gains of pigs and packed cell volume and red blood cell and hemoglobin concentrations of blood from pigs fed two diets.

	Diet[a]	
	SPI	SPP
Average daily gain, g	54.0	54.8
SEM	2.7	2.7
Packed cell volume, %	28.0	31.4
SEM	1.0	1.0
Red blood cell, $x10^6$/ul	4.19	4.60
SEM	0.22	0.22
Hemoglobin, g/dl	8.85	9.65
SEM	0.34	0.34
MCV[b], u^3	68.3	67.5
SEM	1.4	1.4

[a]SPI=diet 2 (SPI) of experiment 2 plus additional soy protein isolate; SPP=SPI plus putrescine (25g/kg dry diet).

[b]mean red cell volume.

day 7 of age in experiment 2).

Absorption measurements:

Regression curves generated from xylose absorption values are depicted in figure 9. Various parameters are reported in table 30. There was a tendency for slopes (B_1), curvatures (B_2), and maximum plasma xylose values to be greatest for the curve representing the xylose absorption in pigs fed SPP suggesting that putrescine enhances xylose absorption in pigs fed soybean protein. Results of both experiments 1 and 2 indicate that xylose absorption was enhanced by the inclusion of putrescine or ethylamine in diets containing soybean proteins. In experiment 2, prolonged administration of soybean protein (greater than 7 days) was necessary to reduce xylose absrption in pigs, but in experiment 3 prolonged administration was not necessary. This difference in response may be due to the greater proportion of soybean protein in the diets of experiment 3. In experiment 2 soybean protein isolate contributed 20% of dietary protein, whereas in this experiment it contributed 50% of the dietary protein.

Mucosal morphology:

Villus length in pigs (Table 31) was not statistically different for the two diets, but was least in the jejunum of pigs fed SPI ($p<.10$). Mean crypt depth (Table 31) was least for SPI ($p<.026$), but this difference tended to occur only in the jejunum and ileum. This result is similar to that for experiment 2 which suggests that SPI reduced the size of the

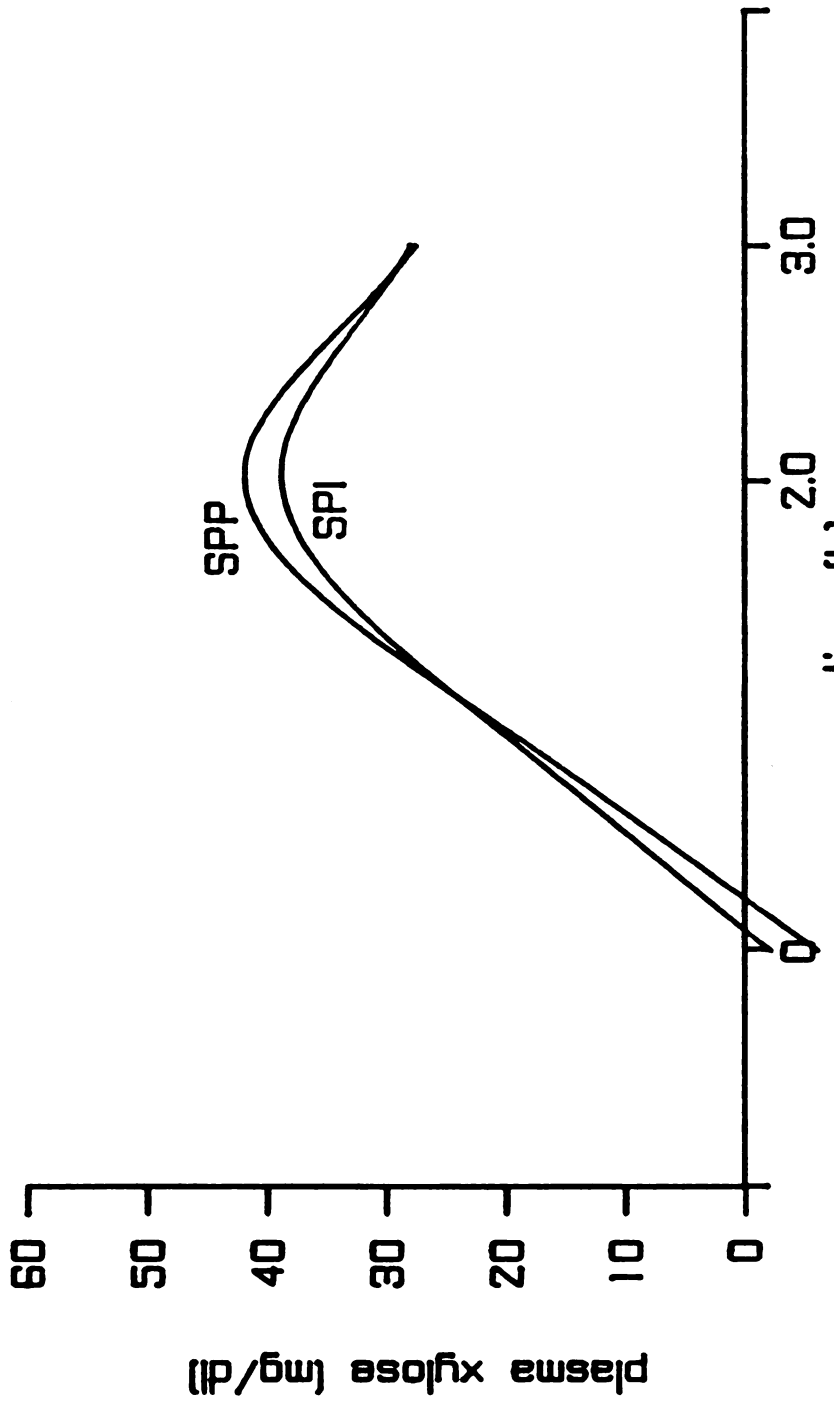


Figure 9. Regression curves generated from xylose absorption curves of pigs fed two diets.

Table 30. Xylose absorption curve parameters for pigs fed two diets.

	Diet	
	SPI	SPP
Maximum plasma xylose, mg/dl	38.9	42.1
SEM	2.0	2.0
Time of maximum plasma xylose,h	2.05	2.13
SEM	0.05	0.05
Slope of curve[a]	38.1	40.1
SEM	2.4	2.4
Curvature of curve[b]	-9.37	-9.63
SEM	0.73	0.73

[a]Slope=B_1, of regression equation for curve.

[b]Curvature=B_2 of regression equation for curve.

Table 31. Villus length, crypt depth, and mitotic index in three sites of the small intestine of pigs fed two diets.

		Diet		Site	
	Site[a]	SPI	SPP	mean	SEM
Villus length, um	1	447	367	407[d]	66
	2	196	532	364[c]	66
	3	256	299	277[d]	66
Treatment mean		300	399		
SEM		54	54		
Crypt depth, um	1	826	795	811	77
	2	419	731	575	77
	3	740	800	770	77
Treatment mean		662[e]	775[f]		
SEM		63	63		
Mitotic index, %	1	1.3	1.5	1.4[h]	0.3
	2	3.3	3.1	3.2[g]	0.2
	3	3.5	2.4	3.0[g]	0.2
Treatment mean		2.7	2.3		
SEM		0.2	0.2		

[a]1=duodenum; 2=jejunum; 3=ileum

[b]Diet X site interaction ($p<.11$).

c differs from d ($p<.10$).

e<f ($p<.026$).

g>h ($p<.01$).

proliferative compartment for enterocytes of the distal small intestine.

Mucosal cytology:

Mitotic indices of the intestinal mucosa (Table 31) were not significantly influenced by diet, but were least in the duodenum ($p<.01$). As in experiment 2 this is not consistent with crypt depth. Number of crypts may have differed among the pigs fed different diets, thus explaining the discrepancy between crypt depth and mitotic index.

Mucosal metabolites:

Small intestinal mucosal protein, DNA, and RNA data are reported in Table 32. Mucosal protein concentration did not differ between diets. In experiment 2, differences in mucosal protein between pigs fed SPP and SPI were not apparent at day 7, but were at day 14. This difference only at 14 days of age suggests that soy protein isolates need to be fed longer than 1 week of age to affect mucosal protein concentration in piglets.

Mucosal RNA and DNA concentrations tended to be greatest in pigs fed SPI. Mucosal DNA was greater in the ileum than in the duodenum ($p<.10$) which is consistent with experiments 1 and 2 in which the distal regions of the small intestines of both calves and pigs had greater concentrations of mucosal DNA than did the proximal regions.

Mucosal protein:DNA tended to be greatest in pigs fed SPP. A significant diet X site interaction existed ($p<.05$).

Table 32. Mucosal protein, RNA, and DNA concentrations and and their ratios in three sites of the small intestine of pigs fed two diets.

		Diet		Site	
	Site[a]	SPI	SPP	mean	SEM
Protein, ug/mg wet mucosa	1	47.27	45.28	46.28	2.24
	2	39.99	38.80	39.39	2.24
	3	40.23	40.71	40.47	2.24
Treatment mean		42.50	41.60		
SEM		1.83	1.83		
RNA, ug/mg wet mucosa	1	4.44	4.08	4.26	0.23
	2	3.88	3.95	3.91	0.23
	3	4.31	4.02	4.17	0.23
Treatment mean		4.21	4.01		
SEM		0.23	0.16		
DNA, ug/mg wet mucosa	1	3.86	3.75	3.81[b]	0.28
	2	4.77	3.30[d]	4.03	0.28
	3	4.69	5.11[e]	4.90[c]	0.28
Treatment mean		4.44	4.06		
SEM		0.26	0.21		
Protein:DNA[f]	1	12.2	11.2[i]	11.7	0.7
	2	8.4	14.2[h]	11.3	0.7
	3	8.6	8.1[g]	8.3	0.7
Treatment mean		9.7	11.2		
SEM		0.6	0.6		
RNA:DNA	1	1.17	1.07	1.12[j]	0.06
	2	0.81	1.16	0.98	0.06
	3	0.92	0.75	0.83[k]	0.06
Treatment mean		0.97	0.99		
SEM		0.06	0.05		
Protein:RNA	1	10.6	10.5	10.6	0.8
	2	10.5	11.8	11.1	0.8
	3	9.5	10.8	10.1	0.8
Treatment mean		10.2	11.0		
SEM		0.7	0.7		

[a]1=duodenum; 2=jejunum; 3=ileum.
[b]b<c ($p<.10$); d<e ($p<.05$).
[f]Diet X site interaction ($p<.05$).
g<h ($p<.02$); g<i ($p<.01$); j>k ($p<.05$).

Protein:DNA of the ileum was less than that of the jejunum ($p<.02$) and duodenum ($p<.01$) of pigs fed SPP. Protein:DNA was greater in the jejunum of pigs fed SPP than in the jejunum of those fed SPI ($p<.05$) indicating that intestinal epithelial cells of the ileum are smaller than those of the duodenum and jejunum as was reported in experiment 2. Furthermore, putrescine increased cell size in the jejunum. Mucosal RNA:DNA was greater in the duodenum than the ileum of all pigs whereas mucosal protein:RNA was not different among sites or diets.

The differences among sites and days in mucosal protein, DNA, and RNA concentrations due to diet are not as large in this experiment as in experiment 2. The differences in experiment 2 tended to increase over age. In this experiment pigs had only been fed diets up to 7 days of age which may not be long enough for any diet effect to become evident. Kilshaw and Slade (1982) reported that calves fed soybean protein for longer times had greater villus atrophy. The same appears to occur in piglets.

Mucosal enzymes:

Total and specific activities of mucosal ornithine decarboxylase (Table 33) were lowest in pigs fed SPP indicating that the amount of putrescine fed to pigs in this experiment inhibited ornithine decarboxylase. These results agree with those of experiment 1 and 2 . However, in this experiment ornithine decarboxylase tended to be greatest in the jejunum rather than the duodenum as was reported in

Table 33. Specific and total activities of mucosal ornithine decarboxylase (ODC), sucrase, and dipeptidase in three sites of the small intestine of pigs fed two diets.

		Diet		Site	
	Site[a]	SPI	SPP	mean	SEM
Specific activity of ODC[u]	1	33.7[d]	2.9	18.3[b]	3.6
	2	59.7[e]	5.2	32.4[c]	3.6
	3	48.9	7.2	28.1	3.6
Treatment mean		47.4[f]	5.1[g]		
SEM		3.0	3.0		
Total activity of ODC[v]	1	1.57	0.12	0.8	0.2
	2	2.37	0.14	1.3	0.2
	3	1.81	0.26	1.0	0.2
Treatment mean		1.92[h]	0.17[i]		
SEM		0.13	0.13		
Specific activity of sucrase[w]	1	193	126	159[j]	12
	2	130	101	115[k]	12
	3	110	90	100[l]	12
Treatment mean		144	106		
SEM		10	10		
Total activity of sucrase[x]	1	9.1	5.8	7.4[m]	0.6
	2	4.1	3.7	3.9[n]	0.6
	3	4.0	3.3	3.6[n]	0.6
Treatment mean		5.7	4.2		
SEM		0.5	0.5		
Specific activity of dipeptidase[y]	1	349	379	364[o]	49
	2	615	895	755[p]	49
	3	501	511	506[q]	49
Treatment mean		488	595		
SEM		40	40		
Total activity of dipeptidase[z]	1	16.1	16.2	16.1[r]	2.2
	2	23.2	32.1	27.7[s]	2.2
	3	18.7	18.4	18.6[t]	2.2
Treatment mean		19.3	22.2		
SEM		1.8	1.8		

[a] 1=duodenum; 2=jejunum; 3=ileum.
b<c ($p<.10$); d<e ($p<.05$); f>g ($p<.008$); h>i ($p<.007$); j>k ($p<.10$); j>l ($p<.02$); m>n ($p<.02$); o<p ($p<.01$); p>q ($p<.02$); r<s ($p<.01$); s>t ($p<.05$).
[u] pmoles CO_2 released/h/mg protein.
[v] pmoles CO_2 released/h/mg wet mucosa.
[w] nmoles sucrose hydrolyzed/h/mg protein.
[x] nmoles sucrose hydrolyzed/h/mg wet mucosa.
[y] nmoles phenylalanylglycine hydrolyzed/min/mg protein.
[z] nmoles phenylalanylglycine hydrolyzed/min/mg wet mucosa.

experiment 2.

Total and specific activities of intestinal brush border sucrase (Table 33) tended to be least in pigs fed SPP. Specific activities were greater in the duodenum than in the jejunum ($p<.10$) and ileum ($p<.02$). Total activities were similiar in that they also were greatest in the duodenum ($p<.01$). This experiment further supports the idea, discussed in experiment 2, that pancreatic proteases degrade brush border enzymes. Sucrase was less for pigs in experiment 3 than those in experiment 2.

Small intestinal cystolic dipeptidase (Table 33) was not significantly influenced by diet, but specific activities were greater in the jejunum than in the duodenum ($p<.01$) and ileum ($p<.02$) and total activities were greater in the jejunum than in the duodenum ($p<.01$) and ileum ($p<.05$). This distribution is consistent with the distribution of peptidase that was observed in pigs of experiment 2 and in an adult pig study (Josefsson and Lindberg, 1965).

SUMMARY AND CONCLUSIONS

In experiment 1, a milk replacer containing 20% of the protein from a soybean protein concentrate did impair small intestinal absorption in preruminant calves when compared to an all-milk protein milk replacer. In addition, supplementing soybean protein with putrescine or ethylamine improved small intestinal absorption. Putrescine or ethylamine prevented the reduction in xylose absorption during xylose absorption tests that was associated with prolonged feeding of soybean protein concentrate. Effects of diet on intestinal villus length and crypt depth were variable. Rates of small intestinal epithelial cell proliferation were reduced in calves fed soybean protein concentrate, but this was corrected by feeding putrescine or ethylamine. Amines also partially negated the reductions in mucosal protein concentrations and efficiencies of protein synthesis associated with feeding soybean protein concentrate to calves up to 2 weeks of age. Amounts of putrescine fed to calves tended to inhibit activities of mucosal ornithine decarboxylase in the proximal jejunum. Soybean protein

concentrate may also inhibit activities of this enzyme in the distal jejunum. Changes in activities of mucosal lactase tended to parallel changes in mucosal protein concentrations and protein :DNA, and RNA:DNA ratios suggesting that soybean protein concentrate may reduce lactase by inhibiting protein synthesis in villus cells. Ornithine decarboxylase may mediate these changes. Soybean protein concentrate may have increased activities of dipeptidase in the cytosol of villus cells by reducing crypt cell proliferation which in turn allowed villus cells a longer time to synthesize enzymes. The limited data on health or growth performance indicate that feeding a soybean protein concentrate milk replacer without putrescine or ethylamine was no different than feeding it with amines or feeding an all-milk protein milk replacer. Fecal consistencies, rectal temperatures, and body weight gains were similiar for all four diets, but ethylamine may have reduced red blood cell numbers and blood hemoglobin concentrations.

In experiment 2, a milk replacer with 20% of the protein from a soybean protein isolate impaired small intestinal absorption in young piglets when compared to an all-milk protein milk replacer. Increases in xylose absorption from 1 to 2 weeks of age in pigs fed an all-milk protein were reduced by inclusion of soybean protein. Addition of putrescine or ethylamine to the soybean protein diet prevented this effect. Crypt depth tended to be least in pigs fed the soybean protein diet alone suggesting that

soybean protein reduces crypt cell proliferation. However, mitotic indices tended to be contradictory to crypt depth measurements and was not influenced by diet. Villus length decreased with advancing age. Mucosal protein, DNA, and RNA concentrations were lowest in pigs fed putrescine and ethylamine, especially at day 14 of age. This suggests that putrescine and ethylamine increase rates of villus cell sloughing. Efficiencies of mucosal protein synthesis were increased with age in pigs fed all-milk protein, soybean protein, or soybean protein plus ethylamine, but were decreased in pigs fed soybean protein plus putrescine. This may explain why specific activities of intestinal cytosolic dipeptidase were least at day 14 of age in pigs fed putrescine. Activities of brush border sucrase were also greater in pigs fed all-milk protein than in pigs fed soybean protein supplemented with putrescine or ethylamine. Concentrations of mucosal putrescine were greatest in the jejunum and ileum of pigs fed putrescine suggesting that the distal regions of the small intestine take up more putrescine than the proximal regions. Soybean protein may also alter the uptake of putrescine, since the distribution of putrescine in the mucosa was influenced by diet. Concentrations of spermidine and spermine were affected by soybean protein and by putrescine suggesting that polyamine biosynthesis was affected. Mucosal ornithine decarboxylase activity was inhibited by putrescine, but was not affected by soybean protein. In addition to the effects on small intestinal variables, packed cell volume, red blood cell and

hemoglobin concentrations, and red cell volumes tended to be reduced in pigs fed ethylamine. Although small intestinal parameters and blood constituents were affected, diet had no effect on body weight.

In experiment 3, adding putrescine to a milk replacer with 50% of the protein from a soybean protein isolate tended to improve small intestinal absorption of xylose during xylose absorption tests in piglets at 1 week of age. Soybean protein alone reduced intestinal crypt depth in the jejunum and ileum suggesting that soybean protein reduces intestinal epithelial cell proliferation. Conversely, mitotic index was not affected by putrescine. Feeding the diets up to 7 days of age was not sufficient to alter mucosal protein concentrations, but concentrations DNA and RNA tended to be least in pigs fed soybean protein without putrescine. Putrescine inhibited mucosal ornithine decarboxylase activities and tended to reduce the activities of brush border sucrase. Diet did not affect intestinal cystolic dipeptidase activities nor did it affect body weight gains up to 7 days of age.

Experiments 1, 2, and 3 were designed with the ideas that soybean protein interferes with polyamine biosynthesis and that supplementing soybean protein with amines would negate any soybean protein effects. Table 34 summarizes the effects that soybean proteins had on small intestinal variables in young piglets and preruminant calves. Soybean protein reduced intestinal absorption, crypt cell

Table 34. Summary of the effects of soybean proteins on small intestinal variables in piglets and calves and the ablity of amines to counteract these effects.

Soybean protein	Counteracted by amines?
Reduced intestinal absorption	Yes
Reduced crypt cell proliferation	Yes
Reduced protein and efficiency of protein synthesis	Partially in calves; reduced further in pigs.
Reduced lactase	No (but amines reduced sucrase and dipeptidase in pigs).
Reduced ornithine decarboxylase in calves only	No (but inhibited further by putrescine).

proliferation, and mucosal protein and efficiency of mucosal protein synthesis in both calves and pigs. Administration of putrescine or ethylamine prevented the reductions in intestinal absorption and crypt cell proliferation in calves and pigs and partially counteracted the reductions in protein and efficiency of protein synthesis in calves. However, amines reduced protein and protein synthesis efficiency even further in pigs. Soybean protein decreased lactase in calves and did not affect sucrase in pigs. Amines had no effect on lactase, but decreased sucrase. Soybean protein decreased ornithine decarboxylase only in calves. Ethylamine did not influence ornithine decarboxylase, but putrescine decreased ornithine decarboxylase in both calves and pigs. The conflicting results of soybean protein and amines on intestinal variables observed in pigs and calves could be due to either a species difference (calves vs pigs) or to a difference in the soybean protein that was fed to the two species of animals (a soybean protein concentrate was fed to calves, but a soy protein isolate was fed to piglets). These differences in response to different soybean proteins further emphasizes the need for studies involving the use of soybean proteins which have been processed differently. Soybean protein had no effect on blood variables, but putrescine reduced packed cell volume in calves and ethylamine reduced packed cell volume, red blood cell concentration and volume, and hemoglobin concentration in piglets.

Future experiments should address two areas. First, performance trials should be designed to determine which

amine to feed and the amount of amine to feed in order to optimize animal health and body weight gains. Ethylamine is less costly and may be favored over putrescine because ethylamine, unlike putrescine, did not inhibit ornithine decarboxylase, the rate-limiting enzyme in the biosynthesis of polyamines.

The second area that future experiments should focus at is the effects of various soybean proteins and amines on small intestinal variables. An understanding of how soybean protein affects the small intestine may enable one to prevent the damages that soybean causes to the intestine. Experiments 1, 2, and 3 have shown that feeding amines may be a way to accomplish this goal. However, some changes in the methods that were employed to measure intestinal variables in experiments 1, 2, and 3 should be considered in future experiments and are discussed below.

Mucosal damage due to dietary constituents causes an increase in intestinal permeability to large molecules (greater than 0.4 nm radius) and a decrease in permeability to smaller molecules (0.4 nm radius or less). Menzies (1984) employed an intestinal absorption method using L-rhamnose as a small probe-marker and lactulose as a large probe-marker. Villus atrophy caused by coeliac disease, tropical malabsorption, acute gastoenteritis, or cytotoxic drug therapy resulted in an increase in the urinary lactulose/L-rhamnose excetion ratio because mucosal surface area and small pore permeation was decreased and large pore permeation

was increased. There is better discrimination between healthy animals and animals with mucosal damage when using the lactulose/L-rhamnose ratio method than when measuring L-rhamnose or D-xylose alone (Menzies, 1984). Therefore, mucosal damage in animals ingesting soybean protein may be detected more easily by using Menzies' differential sugar permeation method than by the D-xylose method used in experiments 1, 2, and 3. Furthermore, increased permeability to larger molecules during mucosal damage may explain the gastrointestinal allergies in humans and animals that are often associated with soybean protein feeding (Barratt and Porter, 1979; Kilshaw and Sissons, 1979a and 1979b; Kilshaw and Slade, 1980).

Noone et al. (1986) has employed urinary lactulose/L-rhamnose ratios to detect changes in intestinal permeability and urinary lactose/lactulose ratios to detect changes in lactose hydrolysis. Noone demonstrated a linear relationship between urinary lactose/lactulose excretion ratios and log jejunal mucosal lactase activity in individuals following ingestion of lactose and lactulose. Sucrose has also been added to the lactulose/L-rhamnose system to measure urinary sucrose/lactulose ratios for a measure of sucrase deficiency (Menzies, 1984). These methods may be used in experiments to supplement or to replace the mucosal disaccharidase assays that were employed in experiments 1, 2, and 3.

Measuring crypt depth and mitotic index as was perfomed in the calf and pig trials does not accurately measure changes in enterocyte proliferation. Indeed, crypt depth and

mitotic index results did conflict in the pig trials. Changes in cell cycle time, crypt cell population, and crypt:villus ratio are not taken into account when only measuring crypt depth and mitotic index. These variables can significantly affect enterocyte proliferation. Al-Mukhtar et al. (1982), Sharp et al. (1982), and Wright (1982) reviewed a method that takes into account many of the kinetic parameters which contribute to the enterocyte proliferation and influx into the villus. A metaphase arresting agent, such as colchicine or vincristine sulfate is first administered to the animal. Then the number of metaphase/crypt is counted in serial biopsies. The crypt cell production rate, expressed as cells produced per crypt per hour, is calculated from a regression line of the accumulated metaphase-blocked cells plotted against time after administration of the metaphase arresting agent. This calculation takes into account the cell cycle time, the fraction of the cell population which is devoted to proliferation, and the crypt cell population. The crypt:villus ratio is also measured and then multiplied by the crypt cell production rate to determine the net villus influx. Conclusions drawn on methods which do not employ all of these measurements may be subject to criticism.

Villus length was measured in experiments 1, 2, and 3 because it has been reported to be affected by soybean protein in calves and piglets (discussed in literature review). One measures villus length as an indirect measure of villus cell population and assumes that all of the villi

are the same shape. In experiments 1, 2, and 3 villus height was highly variable among animals within treatments suggesting that the villi were variable in shape. Correlations between villus height and villus cell population was0.843 in rat intestine with normal morphology, but only 0.619 in intestine with abnormal morphology (Al-Mukhtar et al., 1982). These data discourage the use of villus height as a measure of villus morphology. Villus cells should be counted in microdissected villi in order to prevent discrepancies caused by variable villus shape (Wright, 1982).

In the calf and pig trials, intracellular and extracellular polyamines were not quantitated individually. In future studies, these individual compartments should be examined to determine whether the putrescine or ethylamine that is fed is absorbed by the enterocyte as shown by Kumagai and Johnson (1987) or whether the amines are acting at the level of the cell membrane as suggested by Seidel (1985). Various enzymes of the polyamine biosnthetic pathway should be measured, especially ornithine decarboxylase and S-adenosylmethionine decarboxylase, the two rate-limiting enzymes of the biosynthesis of polyamines. This information would aid in understanding the mechanism of amine action in the intestine. It is also of interest to know whether the amines are acting on the crypt cells or villus cells or both. The target site could lead one to speculate whether the amines affect proliferation of crypt cells only or whether they also affect differentiation of villus cells.

Future experiments designed to incorporate the ideas and

techniques described above may lead us to an understanding of how soybean protein affects intestinal function and how intestinal growth and development may be enhanced. The use of amines to enhance growth and development is not limited to intestinal tissue, but could be applied to other tissues such as bone and muscle.

APPENDICES

Appendix 1. Preparation of intestinal mucosa for brush border lactase and sucrase activity, cytosolic peptide hydrolase and ornithine decarboxylase activity assays.

Reagents:

1. Sodium phosphate buffer, (50 mM, pH 7.2)	68.4 ml 0.5 M Na_2HPO_4 plus 31.6 ml 0.5 M NaH_2PO_4 made to 1000 ml with D-H_2O. Adjust pH to 7.2. Store at room temp.
2. Sodium phosphate buffer, (14% glycerol, 50 mM, pH 7.2)	14 ml glycerol made to 100 with 50 mM sodium phoshate buffer. Mix well. Make up fresh.
3. Physiological saline solution (0.9%)	0.9 g NaCl up to 100 ml with D-H_2O.

Procedure:

Immediately after obtaining the intestinal section from the live animal, flush the section with 100 ml saline and blot it with a paper towel. Scrape off the mucosa with a glass slide and place it in 6 ml of ice cold sodium phosphate- glycerol buffer. Place the buffer and tissue on ice and transport it to the laboratory. Homogenize the buffer and tissue in a centrifuge tube (12 ml, 15x103 mm Nalgene polycarbonate #3117) with a Brinkman Polytron homogenizer on a setting of 7 for 10 seconds. Centrifuge at 1200xg for 10 minutes. Remove 1.0 ml supernatant for the lactase activity assay (store in a small plastic vial at -20 degrees C). Centrifuge the remaining contents at 26000xg for 20 minutes. Remove 1.0 ml

of supernatant for the peptide hydrolase activity assay (store at -20 degrees C) and use the remaining supernatant for the ornithine decarboxylase activity assay (store at -85 degrees C). Discard the precipitate. Protein determination is made on each of the supernatant fractions (using the BioRad protein kit from BioRad which utilizes the Bradford procedure, 1976). Enzyme activity is expressed on a protein basis.

Appendix 2. Lactase activity in intestinal mucosa.

(from Dahlqvist, 1964 and Messer and Dahlqvist, 1966)

Reagents:

1. Sodium phosphate buffer (0.5 M, pH 6.0)	880 ml 0.5 M NaH_2PO_4 plus 120 ml 0.5 M Na_2HPO_4 adjusted to pH 6. Store at room temperature.
2. Peroxidase solution (1.0 mg/ml)	10 mg Horseradish peroxidase (from Sigma Chemical, crude, 45 units/mg solid) made to 100 ml with D-H_2O. Store at -20 degrees C.
3. o-Dianisidine solution (20 mg/ml)	500 mg o-dianisidine-HCL (from Sigma) made to 25 ml with D-H_2O. Store at 4 degrees C.
4. Glucose standard solution (200 mg/l)	200 mg glucose, 0.6 g benzoic acid, 1.2 g sodium benzoate made to 1000 ml with D-H_2O. Store at 4 degrees C.
5. Lactose solution (0.062 M)	2.234 g lactose made to 100 ml with D-H_2O. Make up just before using.
6. Glucose oxidase reagent	62.5 mg glucose oxidase (from Sigma, Type II, 20,000 units/g solid), 0.25 ml peroxidase solution, 0.25 ml dianisidine solution made to 50 ml with 0.5 M sodium

phosphate buffer, pH 6.0. Make up just before using.

7. Sulfuric acid solution (18%) — 18 ml concentrated H_2SO_4 made to 100 ml with D-H_2O. Store at room temperature.

Procedure:

In 12 x 75 mm glass tubes add (in quadruplicates):

100 ul lactose solution

100 ul glucose oxidase reagent

Place tubes in 37 degrees C water bath and add: 20 ul intestinal mucosa sample supernatant (previously prepared). After 15 minutes add 2 ml H_2SO_4 solution to 2 of the 4 tubes. After 75 minutes add 2 ml H_2SO_4 solution to the remaining 2 tubes. Between the 15 and 75 minute times prepare standards as follows (in triplicates):

Glucose solution (ul)	0	5	10	15	20	25	30
Buffer (ul)	120	115	110	105	100	95	90
Glucose/tube (ug)	0	1	2	3	4	5	6

Place standards in water bath, add 100 ul glucose oxidase reagent, incubate for at least 15 minutes, and then add 2 ml H_2SO_4 solution. Read absorbance of final samples on spectrophotometer at 530 nm. Response curve should be linear.

Calculations:

Lactase activity (umoles lactose hydrolyzed/hr/ml sample) = (G75 - G15) x 50/180

where G75 and G15 are the amount of glucose in ug found after 75 and 15 minutes, respectively.

Appendix 3. Sucrase activity in intestinal mucosa.

(from Dahlqvist, 1964 and Messer and Dahlqvist, 1964)

Reagents:

1. Sodium phosphate buffer (0.5M, pH 6.0)	880 ml 0.5M NaH_2PO_4 plus 120 ml 0.5 M Na_2HPO_4 adjusted to pH 6. Store at room temperature.
2. Peroxidase solution (1.0 mg/ml)	10 mg Horseradish peroxidase (from Sigma, crude, 45 units/mg solid)made to 10 ml with D-H_2O. Store at -20 degrees C.
3. o-Dianisidine solution	500 mg o-dianisidine-HCl (from Sigma)made to 25 ml with D-H_2O. Store at 4 degrees C.
4. Glucose standard solution (200 mg/l)	200 ml glucose, 0.6 g benzoic acid,1.2 g sodium benzoate made to 1000 ml with D-H_2O. Store at 4 degrees C.
5. Sucrose solution (0.062M)	2.122g sucrose (from Mallinckrodt)made to 100 ml with D-H_2O.
6. Glucose oxidase reagent	62.5 mg glucose oxidase (from Sigma, Type II, 20,000 units/g solid), 0.25 ml peroxidase solution, 0.25 ml dianisidine solution made to 50 with 0.5 M sodium phosphate buffer, pH 6.0 Make up just before using.

7. Sulfuric acid solution — 18 ml conc. H_2SO_4 diluted to 100 with D-H_2O. Store at room temperature.

Procedure:

I. First the sucrose solution is made glucose-free. 100 mg glucose oxidase (20,000 Sigma units/g solid) plus 20 mg catalase (1600 Sigma units/mg solid) is added to 100 ml of .062M sucrose solution. Two drops of toluene are added and the solution is placed in a 300 ml open-topped flask for 18 hours with occasional swirling. At the end of 18 h., the solution is passed through a layer of TEAE cellulose 3 times. The TEAE cellulose is prepared by stirring 5 g of TEAE cellulose (from Schleicher and Schuell Co., 0.81 meq/g) in 200 ml of 0.2 M sodium bicarbonate buffer, letting sit for 30 min., drawing off the fines with suction, and pouring the remaining contents on Whatman #4 filter paper in a 7 cm Buchner funnel. Whatman paper is placed above the cellulose also. Water is passed through the cellulose until the eluent is at neutral pH. A total of 3 incubations, each followed by cellulose filtering, is conducted on the 100 ml volume of sucrose solution. The glucose-free sucrose solution is frozen in 10 ml aliquots at -20 degrees C.

II. In 12x75 mm glass tubes add (in Quadriplicates):

100 ul glucose-free sucrose solution

100 ul glucose oxidase reagent

Place tubes in 37 degrees C water bath and add: 20 ul thawed intestinal mucosa sample supernatant (previously prepared). After 15 minutes add 2 ml H_2SO_4 solution to 2 of the 4 tubes.

After 75 minutes add 2 ml H_2SO_4 solution to the remaining 2 tubes. Between the 15 minute and 75 minute times prepare standards as follows (in triplicates):

Glucose solution (ul)	0	5	10	15	20	25	30
Buffer (ul)	120	115	110	105	100	95	90
Glucose/tube (ug)	0	1	2	3	4	5	6

Place standards in water bath, add 100 ul glucose oxidase reagent, incubate for at least 15 minutes, and then add 2 ml $H_2 SO_4$ solution. Read absorbance of final samples on spectrophotometer at 530 nm. Standard curve should be linear.

Calculations:

Sucrase activity (umoles sucrose hydrolyzed/hr/ml sample) = (G75 - G15) x 50/180

where G75 and G15 are the amount of glucose in ug found 75 and 15 minutes, respectively.

Appendix 4. Dipeptide hydrolase activity in intestinal mucosa.

(from Nicholson and Kim, 1975)

Reagents:

1. Tri-HCL buffer (50 mM, pH 8.0)	4.44 g Tris-HCl plus 2.65 g Tris-Base made to 1000 ml with D-H_2O. Adjust to pH 8.0. Store at room temperature.
2. Peroxidase solution (1.0 mg/ml)	10 mg Horeradish peroxidase (from Sigma, crude, 45 units/mg solid) made to 10 ml with D-H_2O.Store at -20 degrees C.
3. o-Dianisidine solution (20 mg/ml)	500 mg o-dianisidine-HCL (from Sigma made to 25 ml with D-H_2O. Store at 4 degrees C.
4. Dipeptide solution (L-phenylalanylglycine, 8 mM)	88.88 mg L-phenylalanylglycine (from Sigma) made to 50 ml Tris-HCl buffer. Make up fresh.
5. Phenylalanine solution (0.2 mM)	33.04 mg L-phenylalanine made to 11 ml with D-H_2O. Make up fresh.
6. L-amino acid oxidase reagent (LAOR)	42 mg L-amino acid oxidase (from Sigma, Type I, 0.43 units/mg solid), 2 ml peroxidase solution 0.5 ml dianisidine solution made to 100 ml with Tris buffer.
7. Sulfuric acid solution (50%)	50 ml concentrated H_2SO_4 diluted to 100 with D-H_2O. Store at room temperature.

Procedure:

To two 12 x 75 mm glass tubes add:

0.5 ml dipeptide solution

1.0 ml LAOR

To two more tubes (enzyme blanks) add:

0.5 ml Tris buffer

1.0 ml LAOR

Place tubes in 37 degrees C water bath and add:

10ul intestinal mucosa sample supernatant (previously prepared).

After 30 minutes add 0.74 ml of H_2SO_4 solution.

Prepare standards as follows (in triplicates):

Phenylalanine solution(ul)	0	25	50	100	200	300	400	500
Tris buffer (ul)	500	475	450	400	300	200	100	0
AA/tube (nmoles)	0	5	10	20	40	60	80	100

Then add 10 ul of intestinal mucosa sample buffer in each standard tube, place in water bath, add 1.0 ml LAOR, incubate for at least 20 minutes, then add 0.74 ml H_2SO_4 solution. Read absorbance of final samples on spectrophotometer at 530 nm. Standard curve should be linear.

Calculation:

Peptidase activity (nmoles dipeptide hydrolyzed/min/ml sample = nmol. AA x 100/30

Appendix 5. Ornithine decarboxylase activity in intestinal mucosa.

(from Slotkin and Bartolome, 1983)

1. Pyridoxal 5'-phosphate solution (PLP)	24.7 mg PLP (from Sigma) made to 100 ml with D-H_2O. Make up fresh.
2. Dithiothreitol solution	62 mg dithio.(from Sigma) made to 10 ml with D-H_2O. Make up fresh.
3. Trichloroacetic acid (10%)	10g TCA made to 100 ml with D-H_2O. Store at room temperature.
4. Methylbenzethonium hydroxide (Hyamine hydroxide) (1M)	from Sigma, 1M in methanol.
5. L-(carboxyl-^{14}C) ornithine hydrochloride	from Amersham, 50 uCi/ml.

Procedure:

Prepare vial stoppers (from Kontes, to fit 15 x 52 mm glass scintillation vials when inverted) with center wells (from Kontes) containing 13 x 20 mm wicks cut from Whatman #1 filter paper. Number of vial set-ups = no. of samples x 3. This will allow samples to be analyzed in duplicate with one blank. Make up a mixture of dithiothreitol solution and labelled ornithine in a 10x75 mm glass tube to provide 1.30 ul ornithine and 10 ul dithiothreitol per vial.

To each vial add: 11.3 ul dithiothiothreitol-ornithine mixture

200 ul thawed intestinal mucosa sample supernatant

11.3 ul PLP solution (add last)

Add 0.5 ml 10% TCA to one of the vials to serve as a blank. Place the inverted serum stopper containing the center well and wick over the vial. Place the vials in a 37 degrees C water bath for 30 minutes. At 30 minutes, inject 0.5 ml 10% TCA into the reaction mixture of the 2 vials and 0.2 ml of hyamine hydroxide into the center well containing the wick of all 3 vials. This can be done with a syringe equipped with a 20 guage needle. Inject through the side of the stopper. Replace vials in water bath for an additional 30 minutes. At the end of the incubation period, remove the stopper, wipe the underside of the wick with a paper towel to remove any splash, and cut off the center well into a scintillation vial containing 10 ml of non-aqueous cocktail (from Research Products International Corp., 3a20). Substrate amount is verified by adding 11.3 ul of the dithiothreitol-ornithine mixture to 10 ml of cocktail. All samples are counted twice for 10 minutes in a scintillation counter. Samples are allowed to set over night before counting.

Appendix 6. Preparation of intestinal mucosa for polyamine determination.

Reagents:

1. 10% TCA solution — 10g trichloroactetic acid made to 100 ml with D-H_2O. Store at room temperature.
2. Diethylether, anhydrous [$(CH_3CH_2)_2O$]
3. Physiological saline (0.9%) — 0.9g NaCl made to 100 ml with D-H_2O.

Procedure:

Immediately after obtaining the intestinal section from the live animal, flush the section with 100 ml saline and blot it with a paper towel. Scrape off the mucosa with a glass slide and place it in a centrifuge tube (12 ml, 15x103 mm Nalgene polycarbonate#3117) containing 5 ml 10% TCA solution. Place the tube and contents on ice and transport it to the laboratory. Homogenize the TCA and sample in the tube with a Brinkman Polytron homogenizer on a setting of 7 for 10 seconds. Centrifuge at 26000xg for 10 minutes.Remove the supernatant and place it in a 16x125 mm diposable test tube. Add an equivalent volume of ether, vortex, and let sit for two minutes. Remove the organic layer (top) and discard. repeat the ether extraction step above until the supernatant is near neutral pH. Store the aqueous layer at -20 degrees C for later polyamine determination.

Appendix 7. Determination of polyamine concentrations in intestinal mucosa.

(from Brown et al., personal communication, 1986)

Reagents:

1.Internal standard solution, 1,6-Diaminohexane, 420 um	48.804 mg 1,6-diaminohexane (from Sigma Chem. Co.)made to 1 l with 0.005 N HCl. Store at 5 degrees C.
2.Internal standard solution, 1,6-Diaminohexane, 210 uM	Prepared from 420 uM I.S.
3.External standard solution, Putrescine, 32.8125 uM Spermidine, 13.125 uM Spermine, 32.125 uM	5.286 mg putrescine dihydrochloride plus 7.672 mg dispermidine triphosphate plus 13.069 mg spermine diphosphate from Sigma made to 1l with 0.005N HCl. Store at 5 degrees C.
4. Carbonate buffer, 0.5 M, pH 9.2	0.25 M sodium bicarbonate plus 0.25 M sodium bicarbonate. Adjust to pH 9.2 with bicarbonate. Store at room temp.
5. Potassium carbonate, anhydrous	from Mallinckrodt.

6. Dansyl chloride solution, 2 mg/ml	Dansyl chloride, 100 mg/ml acetone (5-dimethylaminonapthalene-1-sulfonylchloride) (from Pierce Chem. Co.) diluted to 2 mg/ml with distilled acetone. Make up just prior to use.
7. n-Heptane	from Sigma Chem. Co.
8. Methanol	HPLC grade, from Mallinckrodt.
9. Acetic acid, glacial	from Mallinckrodt.
10. Acetonitrite	HPLC grade, from Mallinckrodt.
11. 1-Heptanesulfonic acid, 0.02 M	Four prepackaged vials of PIC-B7 reagent (from Waters Associates) made to 1 l of 5X D-H_2O. Stir for 5 minutes and filter through 0.45 um filter. Make up 1 day before use and store at room temp.
12. Ammonium hydroxide, conc.	from Mallinckrodt.

Derivatization Procedure:

Place 0.5 ml of thawed sample (previously prepared) into a 16x100 mm glass tube. Cap the tube with a rubber stopper equipped with a center well (from Kontes) containing concentrated ammonium hydroxide for approximately 1 minute to

neutralize the sample. To 13x100 mm screw capped culture tubes add:

	calf samples	pig samples
Neutralized sample, ul	200	400
Int. std. (210uM), ul	50	50
Carbonate buffer, ul	300	600
Potassium carbonate, mg	100	200
Dansyl chloride solution, ul	500	1000

Thoroughly mix samples, cap tubes, and allow the mixture to react at room temperature for 16 hours. At the end of 16 hours, add 5 ml of n-heptane to each tube, and vortex for 30 seconds. Remove 5 ml of organic layer, place in a glass tube, and blow to dryness with streams of nitrogen gas. Reconstitute the dried samples as follows:

	Calf		Pig	
	samples	standards	samples	standards
Methanol, ul	100	200	100	400
Acetic acid, ul	5	10	5	20

Store the derivatized sample at -20 degrees C until analyzed by the automated liquid chromatograph.

Chromatography Procedure:

A Waters Associates (Milford, Mass.) high performance liquid chromatograph is used to quantitate the dansylated polyamines. A Waters model 720 System Controller is connected to a model 712 refrigerated Waters Intelligent Sample Processor (WISP), a Waters Model 730 Data Module, which integrates peak areas and quantitates polyamine

concentrations, and two Waters Module 510 Pumps. The WISP is programmed to automatically inject 40 ul of derivitized calf sample or 80 ul of derivitized pig sample. Run time is 36 minutes plus 9 minutes equilibration delay. Total analysis time is 45 minutes. A prepacked 250 mm x 4.6 mm I.D. RSIL C18 HL, 10u particle size, reverse phase column equipped with a guard cartridge (Alltech Associates, Inc., Deerfield, IL) is used to chromatograph the dansylated polyamines. Separations are performed at ambient temperature. The mobile phase consists of a combination of acetonitrile and 1-heptanesulfonic acid. The system controller is programmed so that the pumps create a concave gradient (curve #9, Waters). Gradient parameters are 50% acetonitrile and 50% 1-heptanesulfonic acid at zero minutes. One minute after injection of the sample, via the WISP, the acetonitrile is increased until 22 minutes, at which time the gradient parameters are 80% acetonitrile and 20% 1-heptanesulfonic acid. Parameters remain at these values until 36 minutes at which time the initial conditions are resumed for the 9 minute equilibrium delay. Flow rate is 2 ml/minute throughout the run. Fluorescence is detected with a Waters Model 420-AC Fluorescence Detector equipped with 365 nm excitation and 495 nm emission filters. For this study, linearity was observed for injections containing from 100 to 3000 pmoles. Correlation coeffients for putrescine, spermidine, spermine and diaminohexane were 0.990, 0.991, 0.997, and 0.998, respectively. Figure 10 is a chromatograph generated from this procedure.

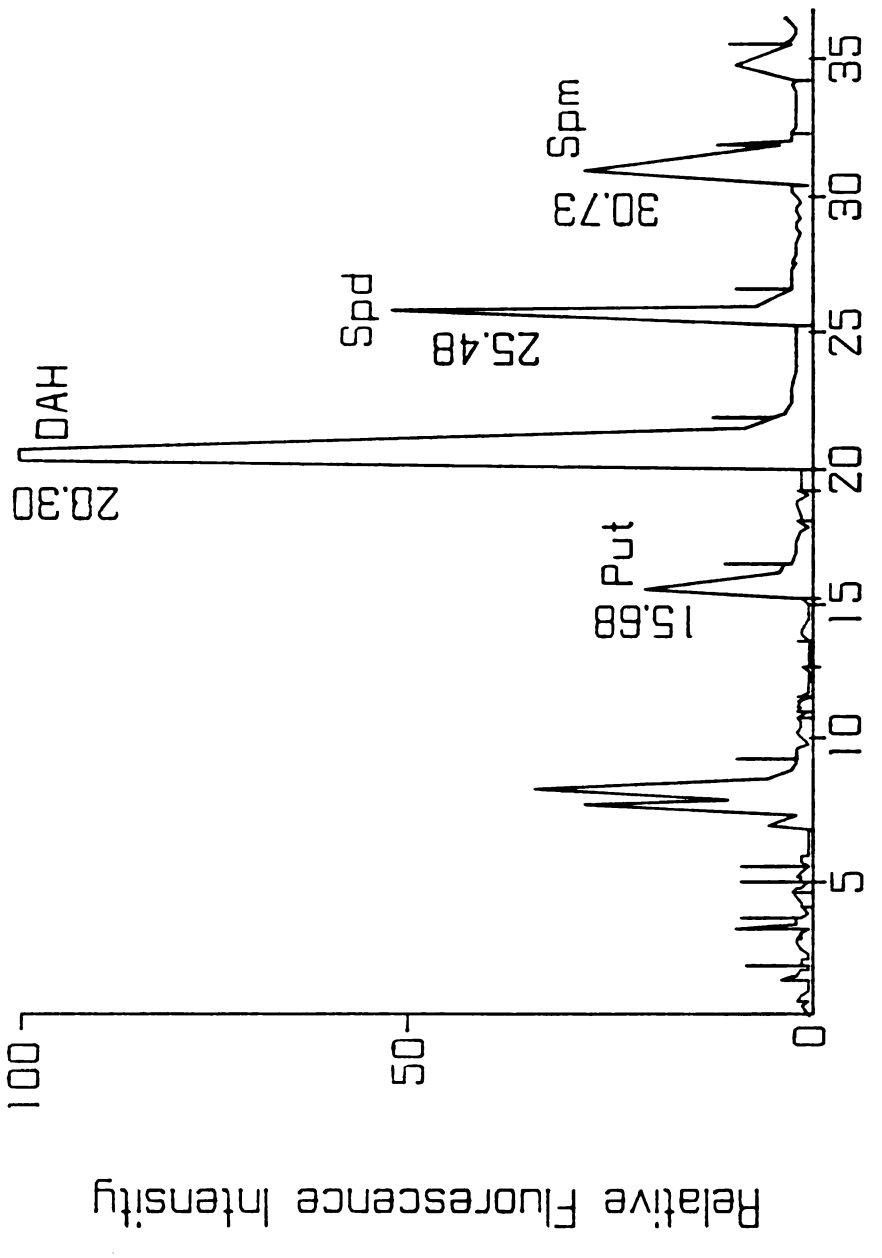


Figure 10. Chromatogram of a standard solution containing 1250 pmoles putrescine (Put), 500 pmoles spermidine (Spd), 1250 pmoles spermine (Spm), and 4000 pmoles 1,6 diaminohexane (DAH).

Appendix 8. Determination of DNA, RNA, and protein in intestinal mucosa.

(Munro, H.N. and A. Fleck, 1966 and Tseng, C. and L.R. Johnson, 1986)

Reagents:

1. 1% Perchloric acid (PCA)	Store at 0-4 degrees C.
2. 2.5% PCA	Store at 0-4 degrees C.
3. 5% PCA	Store at 0-4 degrees C.
4. 10% PCA	Store at 0-4 degrees C.
5. 0.3 N KOH	16.8 g KOH made to 1 liter with D-H_2O. Store at room temp.
6. 1.0 N NaOH	40 g NaOH made to 1 liter with D-H_2O. Store at room temp.
7. 0.1% Ferric chloride solution	1 g $FeCl_3*6H_2O$ made to 1 liter with concentrated (37%) HCl. Store at room temp.
8. Orcinol reagent	1g orcinol (5-methyl resorcinol) monohydrate made to 100 ml with 0.1% $FeCl_3$-HCl solution. Stable for 1 hour--mix with spinbar under hood.
9. Diphenylamine reagent (4%)	4 g diphenylamine made to 100 ml with glacial acetic acid. Make up just prior to use.
10. Acetaldehyde solution	0.4ml diluted to 250ml with H_2O. Store at 4 degrees C.
11. RNA standard (50 ug/ml)	Dissolve 5 mg RNA (Type IV, from Calf Liver, from Sigma) in about

50 ml D-H_2O. Then add conc. (70%) PCA to make a 5% solution in 100 ml and then add D-H_2O up to 100 ml. Make up just prior to use.

12. DNA Standard (50 ug/ml) Dissolve 5 mg DNA (Type I, highly polymerized, from Calf thymus, from Sigma) in about 50 ml D-H_2O, heat is not needed; add conc. (70%) PCA to make a 10% solution in 100 ml and then add D-H_2O up to 100 ml. Make up just prior to use.

Procedure:

1. Homogenize 100 mg of thawed mucosa in 2 ml cold D-H_2O in a 12 ml 15x103 mm tube (Nalgene polycarbonate #3117) with a Brinkman Polytron homogenizer on a setting of 3 for 10 seconds.
2. Add 5 ml cold (4 degrees C) 2.5% PCA. Vortex, let stand in ice for 10-15 min. Vortex again, centrifuge at 35000 x g for 15 min at 4 degrees C.
3. Decant supernatant and discard. Add 5 ml 1% cold PCA, break pellet with glass rod, and centrifuge as above.
4. Decant supernatant and discard. Break pellet and add 4 ml 0.3N KOH. Vortex and incubate at 37 degrees C in H_2O bath for 90 minutes with marbles on tubes. Agitate several times.
5. Remove samples from bath, vortex, and place on ice. When

sample is cold, add 5 ml cold 5% PCA, vortex and replace in ice for at least 15 min.

6. Vortex and centrifuge. Save supernatant in 25 ml graduated tube for the RNA fraction.

7. Wash pellet with 5 ml cold 5% PCA, vortex, centrifuge, and add supernatant to RNA fraction. Collected supernatants are made up to 20 ml with 5% PCA and used for RNA analysis.

8. Store remaining pellet in 4.9 ml of 10% PCA.

9. Mix and vortex tube with pellet, cover tube with marble and incubate at 70 degrees C for 25 min. Mix gently by agitation near beginning, middle and end of incubation period. At end of incubation period, stopper, vortex and place on ice.

10. When cold, centrifuge for 15 min. at 35000 x g. Decant and save supernatant containing DNA into 10 ml graduated tubes.

11. Wash pellet with 4.7 ml cold 10% PCA. Mix , vortex, and centrifuge. Combined supernatants are made up to 10 ml with 10% PCA and used for DNA analysis.

12. Add 3 ml 1.0 N NaOH to the remaining pellet, mix, vortex, and incubate at 55 degrees C for 1 hour agitating frequently.

13. At the end of incubation period make volume to 6 ml with 1.0 N NaOH. Can freeze this protein fraction.

14. For RNA analysis, 2 ml of thawed supernatant (or 1 ml of supernatant and 1 ml 5%PCA) from step 7 are pipetted into 16x125 mm tubes in duplicates. Standard concentrations are 6.25, 12.5, 37.5 and 50 ug/ml and are made up in triplicate as follows:

Concentration(ug/ml)	Vol. of RNA std.(ml)	Vol of 5% PCA(ml)
0	0	2.00
6.25	0.25	1.75
12.5	0.50	1.50
25.0	1.00	1.00
37.5	1.50	0.5
50.0	2.00	0

2 ml of orcinol reagent solution are added to each tube, marbles are placed on tubes, and tubes are incubated in a boiling water bath for 30 minutes. After the incubation period, cool the tubes in running water and then read absorbance at room temp. in a spectrophotometer at 680 nm.

15. For DNA analysis, 2 ml of thawed supernatant (or 1/2 ml of supernatant and 1.5 ml of 10% PCA) from step 10 are pipetted into 16x125 mm tubes in duplicates. Standards are the same concentration as RNA standards and are made in the same manner except that 10% PCA is used rather than 5% PCA. 2 ml of the 4% diphenylamine reagent are added to each tube. Mix, add 0.1 ml acetaldehyde solution to each tube and mix. Place marble on each tube and incubate at 30 degrees C for 16 hours. At end of incubation period, cool to room temp. and read absorbance of all tubes in a spectrophotometer at 595 nm.

16. For protein analysis the Bio-Rad protein kit (from Bio-Rad) is used which utilizes the Bradford procedure (Bradford, 1976). Bovine serum albumin is used as a standard.

Appendix 9. Xylose determination in blood plasma.

(from Frankel,1970, and Bolton, 1976)

Reagents:

1. Perchloric acid (.6M)	57.04 ml $HClO_4$ made to 1000ml with D-H_2O. Store at room temperature.
2. Benzoate diluant	0.6g benzoic acid plus 1.2g sodium benzoate made to 1000ml with H_2O.
3. Xylose standard (50 mg/100 ml)	500 mg xylose (D-xylose practical, from Pfanstiehl Lab.) up to 1000ml with benzoate diluant. Store at 4 degrees C.
4. HCl-$FeCl_3$ solution	2.5 g $FeCl_3$ made to 1000 ml concentrated HCl. Store at room temperature.
5. Orcinol reagent	1g orcinol (5-methyl resorcinol) monohydrate (from Fisher Sci. Co., reagent grade) made to 100 ml with HCl-$FeCl_3$ solution. Stable for 1 hr. Mix with a spinbar under hood.

Procedure:

In centrifuge tubes (Nalgene polycarbonate) add: 1 ml thawed, mixed blood plasma and 1 ml .6 M perchloric acid. Vortex, let sit 5 minutes, and then centurifuge at 27000 x g for 10 minutes. In 25 ml graduated test tubes add (in duplicates):

1.5 ml D-H_2O

0.5 ml protein-free supernatant

2.0 ml orcinol reagent

Place tubes in boiling water bath for 1 hour (do not cover

tubes). Remove tubes, cool in running water, and dilute to 25 ml with D-H_2O. Prepare standards as follows (in duplicate) in 25 ml tubes:

Xylose std. (ul)	0	25	50	100	200	300	400
D-H2O (ml)	2	1.975	1.95	1.90	1.80	1.70	1.60
Xylose in the final 25 ml (mg/100 ml cuvette solution)	0	.05	.1	.2	.4	.6	.8

Add 2 ml orcinol reagent to standards, incubate, and dilute to 25 ml with D-H_2O as with samples. Invert each tube to mix cuvette solution and read on a spectrophotometer at 630 nm. Standard curve should be linear. Calculations: Since the final cuvette solution contains .25 ml plasma the dilution factor is 100. Multiply the concentration read from the standard curve by 100 to obtain actual concentration of xylose (mg %) in the plasma.

BIBLIOGRAPHY

BIBLIOGRAPHY

Adibi, S.A. and Y.S. Kim. 1981. Peptide absorption and hydrolysis. In: L.R. Johnson (Ed.) Physiology of the Gastrointestinal Tract. p.1073. Raven Press, N.Y.

Ahmed, K., S.A. Goueli, and H.G. Williams-Ashman. 1986. Mechanisms and significance of polyamine stimulation of various protein kinase reactions. In: G. Weber (Ed.) Advances in Enzyme Regulation, Vol. 25. p.401. Pergamon Press, N.Y.

Allison, J.B., R. Wannemacher, Jr., and W.L. Banks, Jr. 1963. Influence of dietary protein biosynthesis in various tissues. Fed. Proc. 22:1126.

Al-Mukhtar, M.Y.T., G.R. Sagor, M.A. Ghatei, J.M. Polak, H.S. Koopmans, S.R. Bloom, and N.A. Wright. 1982. The relationship between endogenous gastrointestinal hormones and cell proliferation in models of adaptation. In: J.W.L. Robinson, R.H. Dowling, E.E. Rieken (Ed.) Mechanisms of Intestinal Adaptation. p.243. MTP, Lancaster, UK.

Alpers, D.H. 1986. Uptake and fate of absorbed amino acids and peptides in the mammalian intestine. Fed. Proc. 45:2261.

Alpers, D.H. 1975. The possible role of pancreatic proteases in the turnover of intestinal brush border proteins. Biochim. Biophys. Acta 401:28.

Appleman, R.D. and F.G. Owen. 1975. Breeding, housing, and feeding management. J. Dairy Sci. 58:447.

Aumaitre, A. and T. Corring. 1978. Development of digestive enzymes in the piglet from birth to 8 weeks. II. Intestine and intestinal disaccharides. Nutr. Metab. 22:244.

Ballas, S.K., E.D. Smith, and L.J. Marton. 1985. Effect of polyamines on hemoglobin and globin chain synthesis. Biochem. Biophys. Res. Comm. 129:546.

Barratt, M.E.J. and P. Porter. 1979. Immunoglobulin classes implicated in intestinal disturbances of calves associated with soya protein antigens. J. Immunol. 123:676.

Barratt, M.E.J., P.J. Strachan, and P. Porter. 1978. Antibody

mechanisms implicated in digestive disturbances following ingestion of soya protein in calves and piglets. Clin. Exp. Immunol. 31:305.

Baylin, B., A. Stevens, and K.M. Mohamed Shakir. 1978. Association of diamine oxidase and ornithine decarboxylase with maturing cells in rapidly proliferating epithelium. Biochim. Biophys. Acta 541:415.

Ben-Ghedalia, D., H. Tagari, A. Bondi, and A. Tadmor. 1974. Protein digestion in the intestine of sheep. Br. J. Nutr. 31:125.

Bethell, D.R. and A.E. Pegg. 1981. Polyamines are needed for the differentiation of 3T3-L1 fibroblasts into adipose cells. Biochem. Biophys. Res. Commun. 102:272.

Brown, J.J. and T.W. Perry. 1981. Trypsin and chymotrypsin development in the neonatal lamb. J. Anim. Sci. 52:360.

Brown, N.D., M.P. Strickler, and J.M.Whaun. 1986. A femtomolar ion-pair high performance liquid chromatographic method for determining dansylated polyamine derivatives of red blood cell extracts utilizing an automated polyamine analyzer. Personal communication.

Campos, O.F. and J.T. Huber. 1983. Performance and digestion by calves from limestone added to milk replacers containing soy protein concentrate. J. Dairy Sci. 66:2365.

Campos, O.F., J.T. Huber, and W.G. Bergen. 1982. Partial substitution of milk protein with spray-dried fish solubles or soy protein concentrates in calf milk replacers. J. Dairy Sci. 65:1240.

Catron, D.V., R.O. Baker, and P.A. Hartman. 1957. Enzymes in baby pig nutrition. Proc. of the Ninth Res. Conf., Amer. Meat Inst.

Cheeseman, C.I. 1986. Expression of amino acid and peptide transport systems in rat small intestine. Am. J. Physiol. 251:G636.

Cheeseman, C.I., I. King, and M.W. Smith. 1983. Tissue distribution of exogenous amino acids during transport across the vascularly perfused anuran small intestine. J. Physiol. Lond. 339:133.

Chiu, J. and S.C. Sung. 1972. Effect of spermidine on the activity of DNA polymerases. Biochim. Biophys. Acta 281:535.

Clarke, R.M. 1967. On the constancy of the number of villi in the duodenum of the post-embryonic domestic fowl. J. Embryol. Exp. Morph. 17:131.

Clarke, R.M. 1972. The effect of growth and of fasting on the number of villi and the crypts in the small intestine of the albino rat. J. Anat. 112:27.

Cohen, S.S. 1982. The polyamines as a growth industry. Fed. Proc. 41:3061.

Corring, T., A. Aumaitre, and G. Durand. 1978. Development of digestive enzymes in the piglet from birth to 8 weeks. I. Pancreas and pancrearic enzymes. Nutr. Metab. 22:231.

Corring, T. and D. Bourdon. 1977. Exclusion of pancreatic exocrine secretion from intestine in the pig:Existence of a digestive compensation. J. Nutr. 107:1216.

Dahlqvist, A. 1967. Localization of the small-intestinal disaccharidases. Am. J. Clin. Nutr. 20:81.

Dembinski, A., H. Gregory, S.J. Konturek, and M. Polanski. 1982. Trophic action of epidermal growth factor on the pancreas and gastroduodenal mucosa in rats. In:J.W.L. Robinson, R.H. Dowling, and E.O. Riecken (Ed.) Mechanisms of Intestinal Adaptation. p.281. MTP, Lancaster, U.K.

Dembinski, A.B., T. Yamaguchi, and L.R. Johnson. 1984. Stimulation of mucosal growth by a dietary amine. Am. J. Physiol. 247:G352.

Dollar, A.M. and J.W.G. Porter. 1957. Utilization of carbohydrates by the young calf. Nature. 179:1299.

Dowling, R.H. and B.M. Miazza. 1985. Potentiel d'adaptation du tractus gastrointestinal. Existence d'un axe trophique entero-pancreatique, role des hormones et polyamines. Schweiz. Med. Wschr. 115:963.

Efird, R.C., W.D. Armstrong and D.L. Herman. 1982. The development of digestive capacity in young pigs:Effects of weaning regimen and dietary treatment. J. Anim. Sci. 55:1370.

Ekstrom, K.E., N.J. Benevenga, and R.H. Grummer. 1975. Changes in the intestinal lactase activity in the small intestine of the two breeds of swine from birth to 6 weeks of age. J. Nutr. 105:1032.

El-Nageh, M.M. 1967. Relation entre l'arret de la resorption intestinale des anticorps et le renouvellement de l'epithelium intestinal. Ann. Med. Vet. 111:400.

Emmons, D.B. and E.E. Lister. 1976. Quality of protein in milk replacers for young calves. Factors affecting in vitro curd formation by rennet (chymosin, rennin) from reconstituted skim milk powder. Can. J. Anim. Sci. 56:317.

Emmons, D.B., E.E. Lister, and D.L. Campbell. 1976.Quality of

protein in milk replacers for young calves. III. Rennet coagulation and undenatured whey protein nitrogen content of skim milk powders and commercial milk replacers. Can. J. Anim. Sci. 56:335.

Feuerstein, B.G., N. Pattabiraman, and L.J. Marton. 1986. Spermine-DNA interactions:A theoretical study. Proc. Natl. Acad. Sci. USA. 83:5948.

Fitzpatrick, L.R., P. Wang, B.E. Eikenburg, M.K. Haddox, and R. Johnson. 1986. Effect of refeeding on polyamine biosynthesis in isolated enterocytes. Am. J. Physiol. 250:G709.

Fitzpatrick, L.R., P. Wang, and L.R. Johnson. 1987. Effect of epidermal growth factor on polyamine-synthesizing enzymes in rat enterocytes. Am. J. Physiol. 252:G209.

Fujimoto, M., A. Kanaya, Y. Nakabou, and H. Higihira. 1978. Circadian rhythm in the ornithine decarboxylase activity of rat small intestine. J. Biochem. 83:237.

Fujita, K., Y. Murakami, and S. Hayashi. 1982. A macromolecular inhibitor of the antizyme to ornithine decarboxylase. Biochem. J. 204:647.

Garlich, J.D. and M.C. Nesheim. 1966. Relationship of fractions of soybeans and a crystalline trypsin inhibitor to the effects of feeding unheated soybean meal to chicks. J. Nutr. 88:100.

Garnot, P., J.L. Thapon, C.M. Mathieu, J.L. Maubois, and B. Ribadeau-Dumas. 1971. Determination of rennin and bovine pepsins in commercial rennets and abomasal juices. J. Dairy Sci. 55:1641.

Garnot, P., R. Toullec, J.-L. Thapon, P. Martin, Minh-Thu Hoang, C.-M. Mathieu, and B. Ribadeau Dumas. 1977. Influence of age, dietary protein and weaning on calf abomasal enzymic secretion. J. Dairy Res. 44:9.

Gertler, A., Y. Birk, and A. Bondi. 1967. A comparative study of the nutritional and physiological significance of pure soybean trysin inhibitor and of ethanol-extracted soybean meals in chicks and rats. J. Nutr. 91:358.

Gill, J.L. 1978. Design and Analysis of Experiments in the Animal and Medical Sciences, Vol. 1, 2, and 3. Iowa State Univ. Press, Ames.

Glass, J.R. and W. Gerner. 1986. Polyamine-mediated turnover of ornithine decarboxylase in Chinese-hamster ovary cells. Biochem. J. 236:351.

Glass, J.R. and E.W. Gerner. 1987. Spermidine mediates degradation of ornithine decarboxylase by a non-lysosomal, ubiquitin-independent mechanism. J. Cell. Physiol. 130:133.

Goda, T., K. Yamada, S. Bustamante, and O. Koldovsky. 1983. Dietary- induced rapid decrease of microvillar carbohydrase activity in rat jejunoileum. Am. J. Physiol. 245:G418.

Goda, T., S. Bustamante, and O. Koldovsky. 1985. Dietary regulation of intestinal sucrase and lactase in adult rats:Quantitative comparison of effect of sucrose and lactose. J. Pediatr. Gastroenterol. Nutr. 4:998.

Gorrill, A.D.L. and J.W.G. Nicholson. 1969. Growth, digestibility, and nitrogen retention by calves fed milk replacers containing milk and soybean proteins, supplemented with methionine. Can. J. Anim. Sci. 49:315.

Gorrill, A.D.L., D.J. Schingoethe, and J.W. Thomas. 1968. Proteolytic activity and in vitro enzyme stability in small intestinal contents from ruminants and nonruminants at different ages. J. Nutr. 96:342.

Gorrill, A.D.L. and J.W. Thomas. 1967. Body weight changes, pancreas size and enzyme activity, and proteolytic enzyme activity and protein digestion in intestinal contents from calves fed soybean and milk protein diets. J. Nutr. 92:215.

Gorrill, A.D.L., J.W. Thomas, W.E. Stewart, and J.L. Morrill. 1967. Exocrine pancreatic secretion by calves fed soybean and milk protein diets. J. Nutr. 92:86.

Gosule, L.C. and J.A. Schellman. 1976. Compact form of DNA induced by spermidine. Nature 259:333.

Gray, G.M. 1981. Carbohydrate absorption and malabsorption. In:L.R. Johnson (Ed.) Physiology of the Gastrointestinal Tract. p.1063. Raven Press, N.Y.

Gray, G.M. and N.A. Santiago. 1966. Disaccharide absorption in normal and diseased human intestine. Gastroenterology. 51:489.

Guilloteau, P., T. Corring, R. Toullec, and J. Robelin. 1984. Enzyme potentialities of the abomasum and pancreas of the calf. I.Effect of age in the preruminant. Reprod. Nutr. Develop. 24:315.

Guilloteau, P., R. Toullec, P. Patureau-Mirand, and J. Prugnaud. 1981. Importance of the abomasum in digestion in the preruminant calf. Reprod. Nutr. Develop. 21:885.

Hannonen, P., J. Janne, and A. Raina. 1972. Partial purification and characterization of spermine synthase from rat brain. Biochem. Biophys. Acta 289:225.

Hartman, P.A., V.W. Hays, R.O. Baker, L.H. Neagle, and D.V. Catron. 1961. Digestive enzyme development in the young pig. J. Anim. Sci. 20:114.

Hays, V.W., V.C.Speer, P.A. Hartman, and D.V. Catron. 1959. The effect of age and supplemental amino acids on the utilization of milk and soya protein by the young pig. J. Nutr. 69:179.

Heby, O. and L. Lewan. 1971. Putrescine and polyamines in relation to nucleic acids in mouse liver after partial hepatectomy. Virchows Arch. Abt. B. Zellpath. 8:58.

Heby, O., L.J. Marton, C.B. Wilson, and H.M. Martinez. 1975. Polyamine metabolism in a rat brain tumor cell line: Its relationship to the growth rate. J. Cell. Physiol. 511:22.

Heller, J.S. and E.S. Canellakis. 1981. Cellular control of ornithine decarboxylase activity by its antizyme. J. Cell. Physiol. 107:209.

Henning,S.J. 1985. Ontogeny of enzymes in the small intestine. Ann. Rev. Physiol. 47:231.

Holtta, Erkki, and P. Pohjanpelto. 1986. Control of ornithine decarboxylase in Chinese hamster ovary cells by polyamines:Translational inhibition of synthesis and acceleration of degradation of the enzyme by putrescine, spermidine, and spermine. J. Biol. Chem. 261:9502.

Hooks, R.D., V.W. Hays, V.C. Speer, and J.T. McCall. 1965. Effect of raw soybeans on pancreatic enzyme concentrations and performance of pigs. J. Anim. Sci. 24:894.

Hosomi, M., S.M. Smith, G.M. Murphy, and R.H. Dowling. 1984. Polyamine distribution in the rat intestinal mucosa. Clin. Sci. 66:60P.

Huber, J.T. and O.F. Campos. 1982. Enzymatic hydrolysate of fish, spray-dried fish solubles, and soybean protein concentrate in milk replacers for calves. J. Dairy Sci. 65:2351.

Huber, J.T., N.L. Jacobson, and R.S. Allen. 1961. Digestive enzyme activities in the young calf. J. Dairy Sci. 44;1494.

Huber, J.T., R.J. Rifkin, and J.M. Keith. 1964. Effect of level of lactose upon lactase concentrations in the small intestines of young calves. J. Dairy Sci. 47:789.

Huber,J.T., A.G. Silva, O.F. Campos, andC.M. Mathieu. 1984. Influence of feeding different amounts of milk on performance, health, and absorption capability of baby calves. J. Dairy Sci. 67:2957.

Inoue, H., Y. Konishi, Y. Takeda, and A. Cihak. 1981. The role of putrescine in modulation of DNA synthesis in regeneration liver of rats pretreated with 5-azacytidine. J. Biochem. 89:861.

Janne, J., H. Poso, and A. Raina. 1978. Polyamines in rapid growth and cancer. Biochim. Biophys. Acta 473:241.

Jenkins, K.J., S. Mahadavan, and D.B. Emmons. 1980. Susceptibility of proteins used in calf milk replacers to hydrolysis by various proteolytic enzymes. Can. J. Anim. Sci. 60:907.

Johnson, L.R. 1982. Role of gastrointestinal peptides in intestinal adaptation. In: J.W.L. Robinson, R.H. Dowling, and E.O. Riecken (Ed.) Mechanisms of Intestinal Adaptation. p. 201. MTP, Lancaster, U.K.

Jones, E.E. 1984. Biological development and nutritional requirement of the neonatal pig. Proc. Geo. Nutr. Conf. p.145.

Jones, E.E., J.A. Coalson and J.G. Lecce. 1977. Soy flour as a source of protein for baby pigs. J. Anim. Sci. 45:1073.

Josefsson, L. and T. Lindberg. 1965. Intestinal dipeptidases II. Distribution of dipeptidases in the small intestine of the pig. Biochim. Biophys. Acta 105:162.

Kameji, T. and A.E. Pegg. 1987a. Inhibition of translation of mRNAs for ornithine decarboxylase and S-adenosylmethionine decarboxylase by polyamines. J. Biol. Chem. 262:2427.

Kameji, T. and A.E. Pegg. 1987b. Effect of polyamines on the translation of mRNAs for ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase (ADOMETDC) in reticulocyte lysates. Fed. Proc. 46:1454.

Kanamoto, R., K. Utsunomiya, T. Kameji, and S. Hayashi. 1986. Effects of putrescine on synthesis and degradation of ornithine decarboxylase in primary cultured hepatocytes. Eur. J. Biochem. 154:539.

Kato, Y., H. Nishihara, H. Inoue, E. Gohga, and Y. Takeda. 1978. Polyamine metabolism and DNA synthesis in regenerating rat liver. J. Biochem. 84:1617.

Kedinger, M., P.M. Simon, F. Raul, J.F. Grenier, and K. Haffen. 1982. Effect of various hormones and dietary sugars on the stimulation of intestinal brush-border enzymes in organ culture. In: J.W.L. Robinson, R.H. Dowling, E.O. Riechen (Ed.) Mechanisms of Intestinal Adaptation. p.285. MTP, Lancaster, U.K.

Kilshaw, P.J. and J.W. Sissons. 1979a. Gastrointestinal allergy to soybean protein in preruminant calves. Antibody production and digestive disturbances in calves fed heated soybean flour. Res. Vet. Sci. 27:361.

Kilshaw, P.J. and J.W. Sissons. 1979b. Gastrointestinal allergy to soyabean protein in preruminant calves. Allergenic constituents of soybean products. Res. Vet. Sci. 27:366.

Kilshaw, P.J. and H. Slade. 1980. Passage of ingested protein into the blood during gastrointestinal hypersensitivity reactions:Experiments in the preruminant calf. Clin. Exp. Immunol. 41:575.

Kilshaw, P.J. and H. Slade. 1982. Villus atrophy and crypt elongation in the small intestine of the preruminant calves fed with heated soyabean flour or wheat gluten. Res. Vet. Sci. 33:305.

King, I.S., J.Y.F. Paterson, M.A. Peacock, M.W. Smith, and G. Syme. 1983. Effect of diet upon enterocyte differentiation in rat jejunum. J. Physiol. Lond. 344:465.

Kingsnorth, A.N., M. Abu-Khalaf, G.M. LaMuraglia, P. McCann, K.A. Diekema, J.S. Ross, and R.A. Malt. 1986. Inhibition of ileal and colonic ornithine decarboxylase activity by alpha-difluoromethylornithine in rats:Transient atrophic changes and loss of postresectional adaptive growth. Surgery 99:721.

Kingsnorth, A.N., W.W.K. King, K.A. Diekema, P.P. McCann, J.S. Ross, and R.A. Malt. 1983. Inhibition of ornithine decarboxylase with 2-difuoromethylornithine:Reduced incidence of dimethylhydrazine-induced colon tumors in mice. Cancer Res. 43:2545.

Koldovsky, O., S. Bustamante, K. Yamada. 1982. Adaptability of lactase and sucrase activity in jejuno-ileum of adult rats to changes in intake of starch, sucrose, lactose, glucose, fructose, and galactose. In: J.W.L. Robinson, R.H. Dowling, E.O. Riecken (Ed.) Mechanisms of Intestinal Adaptation. p.153. MTP, Lancaster, U.K.

Koldovsky, O., P. Sunshine, N. Kretchmer. 1966. Cellular migration of intestinal epithelia in suckling and weaned rats. Nature 212:1389.

Kuehn, G.D., and V.J. Atmar. 1982. Posttranslational control of ornithine decarboxylase by polyamine-dependent protein kinase. Fed. Proc. 41:3078.

Kumagai, J. and L.R. Johnson. 1987. Characteristics of putrescine uptake by isolated rat enterocytes. Fed. Proc. 46:1080.

Larson, L.L., F.G. Owen, J.L. Albright, R.D. Appleman, R.C.

Lamb, and L.D. Muller. 1977. Guidelines toward more uniformity in measuring and reporting calf experimental data. J. Dairy Sci. 60:989.

Lichtenberger, L.M., L.A. Graziani, and W.P. Dubinsky. 1982. Importance of dietary amines in meal-induced gastrin release. Am. J. Physiol. 243:G341.

Lindemann, M.D., S.G. Cornelius, S.M. El Kandelgy, R.L. Moser, and J.E. Pettigrew. 1986. Effect of age, weaning and diet on digestive enzyme levels in the piglet. J. Anim. Sci. 62:1298.

Linden, M., Anehus, S., E. Langstrom, B. Baldetorp, and O. Heby. 1985. Cell cycle phase-dependent induction of ornithine decarboxylase-antizyme. J. Cell. Physiol. 125:273.

Lister, E.E. and D.B. Emmons. 1976. Quality of protein in milk replacers for young calves. II. Effects of heat treatment of skim milk powder and fat levels on calf growth, feed intake and nitrogen balance. Can. J. Anim. Sci. 56:327.

Lorenze-Meyer, H., J.A. Volker, N. Friedel, and A. Scheuer. 1982. Investigations of the trophic action of gastrin and cholecystokinin on structure and function of the rat jejunal mucosa. In: J.W.L. Robinson, R.H. Dowling, E.O. Riecken (Ed.) Mechanisms of Intestinal Adaptation. p.227. MTP, Lancaster, U.K.

Loser, C., D. Mustroph, P. Cantor, U. Wunderlich, and U.R. Folsch. 1987. Pancreatic polyamine concentrations and CCK plasma levels in rats after feeding raw soybean flour. Digestion 38:37.

Luk, G.D. and S.B. Baylin. 1982. Ornithine decarboxylase in intestinal maturation, recovery and adaptation. In: J.W.L. Robinson, R.H. Dowling, and E.E. Riecken (Ed.) Mechanisms of Intestinal Adaptation. p.65. MTP, Lancaster, U.K.

Luk, G.D. and S.B. Baylin. 1983. Polyamines and intestinal growth-increased polyamine biosynthesis after jejunectomy. Am. J. Physiol. 245:G656.

Luk, G.D., L.J. Marton, and S.B. Baylin. 1980. Ornithine decarboxylase is important in intestinal mucosal maturation and recovery from injury in rats. Science 210:195.

Magee, D.F. and A.F. Dalley, II. 1986. The gut and the diffuse endocrine system. In: D.F. Magee, and A.F. Dalley,II. (Ed.) Digestion and the Structure and Function of the Gut. p.303. Karger, S. Switzerland.

Magee, D.F. and A.F. Dalley,II. 1986. The pancreas. In: D.F. Magee and A.F. Dalley,II. (Ed.) Digstion and the Structure and the Function of the Gut. p.153. Karger, S. Switzerland.

Magee, D.F. and A.F. Dalley, II. 1986. Absorption. In: D.F. Magee and A.F. Dalley, II (Ed.) Digestion and the Structure and Function of the Gut. p.211. Karger,S. Switzerland.

Malo, C. and D. Menard. 1982. Influence of epidermal growth factor on the development of suckling mouse intestinal mucosa. Gastroenterology 83:28.

Mamont, P.S., P. Bohlen, P.P. McCann, P. Bey, F. Schuber, and C. Tardiff. 1976. Alpha-methylornithine, a potent competitive inhibitor of ornithine decarboxylase blocks proliferation of rat hepatoma cells in culture. Proc. Natl. Acad. Sci. USA. 73:1626.

Mamont, P.S. and C. Danzin. 1981. In vitro and in vivo regulation of S-adenosyl-L-methionine decarboxylase by polyamines. Adv. Polyamine Res. 3:123.

Manners, M.J. and J.A. Stevens. 1972. Changes from birth to maturity in the pattern of distribution of lactase and sucrase activity in the mucosa of the small intestine of pigs. Br. J. Nutr. 28:113.

Matte, J.J., C.L. Girard, J.R. Seoane, and G. J. Brisson. 1982. Absorption of colostral immunoglobulin G in the newborn dairy calf. J.Dairy Sci. 65:1765.

Maudsley, D.V., J. Leif, and Y. Kobayashi. 1976. Ornithine decarboxylase in rat small intestine: Stimulation with food or insulin. Am. J. Physiol. 231:1557.

McCormick, R.J. and W.E. Stewart. 1967. Pancreatic secretion in the bovine calf. J. Dairy Sci. 50:568.

McNiel, N.O., B.E. Eikenburg, and L.R. Johnson. 1987. Role of ornithine decarboxylase in functional development of rat gastric mucosa. Am. J. Physiol. 252:G466.

Menge, H., J.W.L. Robinson, and E.O. Riecken. 1982. Structural and functional correlations in the hypoplastic and hyperplastic mucosa of the rat small intestine. In: J.W.L. Robinson, R.H. Dowling, E.O. Riecken (Ed.) Mechanisms of Intestinal Adaptation. p.383. MTP, Lancaster, U.K.

Menzies, I.S. 1984. Transmucosal passage of inert molecules in health and disease. In: E. Skahauge, K. Heintz (Ed.) Intestinal Absorption and Secretion. p.527. MTP, Lancaster, U.K.

Menzies, I.S. 1974. Absorption of intact oligosaccharide in health and disease. Biochem. Soc. Trans. 2:1042.

Merritt, A.M. and J.H. Reed. 1980. Gastrointestinal function testing. In: N.V. Anderson (Ed.) Veterinary

Gastroenterology. p. 252. Lea and Febiger, Phila., PA.

Metcalf, B.W., P. Bey, C. Danzin, M.J. Jung, P. Casara, and J.P. Ververt. 1978. Catalytic irreversible inhibition of mammalian ornithine decarboxylase (E C 4.1.17) by substrate and product analogues. J. Amer.Chem. Soc. 100:2551.

Minami, H., K. Miyamoto, Y. Fujii, Y. Nakabou, and H. Hagihira. 1985. Induction of intestinal ornithine decarboxylase by single amino acid feeding. J. Biochem. 98:133.

Moon, H.W. 1971. Epithelial cell migration in the alimentary mucosa of the suckling pig. P.S.E.B.M. 137:151.

Moon, H.W. and D.D. Joel. 1975. Epithelial cell migration in the small intestine of sheep and calves. Am. J. Vet. Res. 36:187.

Moon, H.W. and S.M. Skartvedt. 1975. Effect of age on epithelial cell migration in small intestine of chickens. Am. J. Vet. Res. 36:213.

Moore, P. and M.E. Swendseid. 1983. Dietary regulation of the activities of ornithine decarboxylase and S-adenosylmethionine decarboxylase in rats. J. Nutr. 113:1927.

Morrill, J.L., N.L. Jacobson, A.D. McGilliard, and D.K. Hotchkiss. 1965. Use of the re-entrant ileal fistula to study carbohydrate utilization by the young bovine. J. Nutr. 85:429.

Nitsan, Z., R. Volcani, S. Gordin, and A. Hasdai. 1971. Growth and nutrient utilization by calves fed milk replacers containing milk or soybean protein-concentrate heated to various degrees. J. Dairy Sci. 54:1294.

Noone, C., I.S. Menzies, J.E. Banatvala, and J.W. Scopes. 1986. Intestinal permeability and lactose hydrolysis in human rotaviral gastroenteritis assessed simultaneously by non-invasive differential sugar permeation. Eur. J. Clin. Invest. 16:217.

Oka, T., J.W. Perry, and N. Terada. 1982. The regulatory function of spermidine in hormonal control of the development of mouse mammary gland in culture. Fed. Proc. 41:3073.

Osborne, D.L. and E.R. Seidel. 1987. Microfloral polyamines affect mucosal adaptation to colonic obstruction. Fed. Proc. 46:1232.

Owsley, W.F., D.E. Orr, Jr., and L.F. Tribble. 1986. Effects of age and diet on the development of the pancreas and the synthesis and secretion of pancreatic enzymes in the young pig. J. Anim. Sci. 63:497.

Paulin, L. 1986. The effects of 1-aminooxy-3-aminopropane and S-(5'-deoxy-5'-adenosyl)methylthioethyl-hydroxylamine on ornithine decarboxylase and S-adenosyl-L-methionine decarboxylase from Escherichia coli. FEBS Lett. 202:323.

Pearse, A.G.E. and E.O. Riechen. 1967. Histology and cytochemistry of the cells of the small intestine, in relation to absorption. Br. Med. Bull. 23:217.

Pegg, A.E., C. Conover, and A. Wrona. 1978. Effects of aliphatic diamines on rat liver ornithine decarboxylase activity. Biochem. J. 170:651.

Pegg, A.E., I. Matusui, J.E. Seely, M.L. Pritchard, and H. Poso. 1981. Formation of putrescine in rat liver. Med. Biol. 59:327.

Pegg, A.E., K.A. McGovern, and L.Wiest. 1987. Decarboxylation of alpha-difluoromethylornithine by ornithine decarboxylase. Biochem.J. 241:305.

Pegg, A.E., and P.P. McCann. 1982. Polyamine metabolism and function. Am. J. Physiol. 243:C212.

Pegg, A.E., J.E. Seely, H. Poso, F.D. Ragione, and I.S. Zagon. 1982. Polyamine biosynthesis and interconversion in rodent tissues. Fed. Proc. 41:3065.

Pekas, J.C. 1966. Zinc-65 metabolism:Gastrointestinal secretion by the pig. Am. J. Physiol. 211:407.

Pekas, J.C., V.W. Hays, and A.M. Thompson. 1964. Exclusion of the exocrine pancreatic secretion: Effect on the digestibility of the soybean and milk protein by baby pigs at various ages. J. Nutr. 82:277.

Pingoud, A., C. Urbanke, J. Alves, H.J. Ehbrecht, M. Zabeau, and C. Gualerzi. 1984. Effect of polyamines and basic proteins on cleavage of DNA by restriction endonucleases. Biochem. 23:5697.

Porter, J.W.G. and W.B. Hill. 1964. Nitrogen balance trials with calves given synthetic milk diets. Nat. Inst. Res. Dairying Ann. Rept. p.124.

Poso,H., and A.E. Pegg. 1982. Effect of alpha-difluoromethylornithine on polyamine and DNA synthesis in regenerating rat liver: Reversal of inhibition of DNA synthesis by putrescine. Biochem. Biophys. Acta 696:179.

Rackis, J.J. 1965. Physiological properties of soybean trypsin inhibitors and their relationship to hypertrophy and growth inhibition of rats. Fed.Proc. 24:1488.

Richardson, R.I. and A.R.P. Jouan. 1986. The distribution of peptidase activity in the small intestine of sheep. Br. J. Nutr. 55:149.

Robinson, D.W. 1969. The cellular response of porcine skeletal muscle to prenatal and neonatal stress. Growth 33:231.

SAS Institute, Inc. 1982. SAS Users Guide. Cary, N.C.

Schingoethe, D.J., S.D. Aust, and J.W. Thomas. 1970. Separation of a mouse growth inhibitor in soybeans from trypsin inhibitors. J. Nutr. 100:739.

Schingoethe, D.J. and J.W. Thomas. 1969. Pancreatic proteolytic enzymes and growth in rats fed soybean or milk proteins. P.S.E.B.M. 112:453.

Schneider, D.L. and H.P. Sarett. 1969. Growth of baby pigs fed infant soybean formulas. J. Nutr. 98:279.

Seegraber, F.J. and J.L. Morrill. 1979. Effect of soy protein on intestinal absorption ability of calves by the xylose absorption test. J. Dairy Sci. 62:972.

Seegraber, F.J. and J.L. Morrill. 1982. Effect of soy protein on calves' intestinal absorptive ability and morphology determined by scanning electron microscopy. J. Dairy Sci. 65:1962.

Seegraber, F.J. and J.L. Morrill. 1986. Effect of protein source in calf milk replacers on morphology and absorptive ability of small intestine. J. Dairy Sci. 69:460.

Seidel, E.R. 1986. Hormonal regulation of postprandial induction of gastrointestinal ornithine decarboxylase activity. Am. J. Physiol. 251:G460.

Seidel, E.R., M.K. Haddox, and L.R. Johnson. 1984. Polyamines in the response to intestinal obstruction. Am J. Physiol. 246:G649.

Seidel, E.R., M.K. Haddox, and L.R. Johnson. 1985. Ileal mucosal growth during intraluminal infusion of ethylamine or putrescine. Am. J. Physiol. 249:G249.

Seiler, N., F.N. Bolkenius, and O.M. Rennert. 1981. Interconversion catabolism and elimination of the polyamines. Med. Biol. 59:334.

Sepulveda, F.V., K.A. Burton, G.M. Clarkson, and G. Syme. 1982. Cell differentiation and L-ornithine decarboxylase activity in the small intestine of rats fed low and high protein diets. Biochim. Biophys. Acta 716:439.

Sharp, J.G., M.Y.T. Al-Mukhtar, and N.A. Wright. 1982. Evaluation of methods of assessing the intestinal epithelial proliferative response in adaptive situations. Scand. J. Gastro. 17 Suppl.(71-76):163.

Shinki, T., T. Kadofuku, T. Sato, and T. Suda. 1986. Spermidine N1-acetyltransferase has a larger role than ornithine decarboxylase in 1 alpha, 25-dihydroxyvitamin D3-induced putrescine synthisis. J. Biol. Chem. 261:11712.

Silva, A.G. Da. 1984. Factors affecting utilization of soybean proteins included in milk replacers for young calves. Ph.D. Michigan State University, E. Lansing, MI.

Silva, A.G., J.T. Huber, and R.M. Degregorio. 1986a. Influence of substituting two types of soybean protein for milk protein on gain and utilization of milk replacers in calves. J. Dairy Sci. 69:172.

Silva, A.G., J.T. Huber, T.H. Herdt, R. Holland, R.M. Degregorio, and T.P. Mullaney. 1986b. Morphological alterations of small intestinal epithelium of calves caused by feeding soybean protein. J. Dairy Sci. 69:1387.

Sissons, J.W. and R.H. Smith. 1976. The effect of different diets including those containing soya-bean products, on digesta movement and water and nitrogen absorption in the small intestine of the preruminant calf. Br. J. Nutr. 36:421.

Slotkin, T.A. and J. Bartolome. 1986. Role of ornithine decarboxylase and the polyamines in nervous system development:A review. Brain Res. Bull. 17:307.

Smeaton, T.C. and M.W. Simpson-Morgan. 1985. Epithelial cell renewal and antibody transfer in the intestine of the foetal and neonatal lamb. Aust. J. Exp. Biol. Med. Sci. 63:41.

Steglich, C. and I.E. Scheffler. 1982. An ornithine decarboxylase-deficient mutant of Chinese hamster ovary cells. J. Biol. Chem. 257:4603.

Symons, L.E.A. and W.O. Jones. 1966. The distribution of dipeptidase activity in the small intestine of the sheep (Ovis aries). Comp. Biochem. Physiol. 18:71.

Tabata, K. and L.R. Johnson. 1986a. Ornithine decarboxylase and mucosal growth in response to feeding. Am. J. Physiol. 251:G270.

Tabata, K. and L.R. Johnson. 1986b. Mechanism of induction of mucosal ornithine decarboxylase by food. Am. J. Physiol. 251:G370.

Tabor, C.W. and H. Tabor. 1984. Polyamines. Ann. Rev. Biochem. 53:749.

Ternouth, J.H., J.H.B. Roy and S.M. Shotton. 1976. Concurrent studies of the flow of digesta in the duodenum and of exocrine pancreatic secretion of calves. 4.The effect of age. Br. J. Nutr. 36:523.

Ternouth, J.H., J.H.B. Roy and R.C. Siddons. 1974. Concurrent studies of the flow of digesta in the duodenum and of exocrine pancreatic secretion of calves. 2. The effects of addition of fat to skim milk and of "severe" preheating treatment of spray-dried skim-milk powder. Br. J. Nutr. 31:13.

Ternouth, J.H., J.H.B. Roy, S.Y. Thompson, J.Toothill, C.M. Gillies, and J.D. Edwards-Webb. 1975. Concurrent studies of the flow of digesta in the duodenum and of exocrine pancreatic secretion of calves. 3. Further studies on the addition of fat to skim milk and the use of non-milk proteins in milk-substitute diets. Br. J. Nutr. 33:181.

Thornburg, W., J. Grimes, T. Goda, S. Bustamante, P.Pollack, and O. Koldovsky. 1987. Response of activity of jejunal disaccharidases and pancratic amylase in young and middle-aged rats to a high carbohydrate diet. J. Nutr. 117:63.

Thornberg, W., L. Matrisian, B. Magun, and O. Koldovsky. 1984. Gastrointestinal absorption of epidermal growth factor in suckling rats. Am. J. Physiol. 246:G80.

Toullec, R., P. Guilloteau, P. Patureau-Mirand, and J.W. Sissons. 1983. Digestion and absorption of protein in the preruminant. In: European Association for Animal Production. 4th International Symposium. Vol I. p.246.

Trier, J.S. and J.L. Madara. 1981. Functional morphology of the mucosa of the small intestine. In: L.R. Johnson (Ed.) Physiology of the Gastrointestinal Tract.p.925. Raven Press, N.Y.

Tyms, A.S. 1986. Polyamine inhibitors. J. Antimicrobial Chemotherapy 17:131.

Walker, W.R., C.V. Maxwell, F.N. Owens, and D.S. Buchanan. 1986. Milk versus soybean protein sources for pigs: I.Effects on performance and digestibility. J. Anim. Sci. 63:505.

Walsh, J.H. 1981. Endocrine cells of the digestive system. In: L.R. Johnson (Ed.) Physiology of the Gastrointestinal Tract. p.59. Raven Press, N.Y.

Webb, K.E., Jr. 1986. Amino acid and peptide absorption from the gastrointestinal tract. Fed. Proc. 45:2268.

Williams, V.J., J.H.B. Roy, and C.M. Gillies. 1976. Milk substitute diet composition and abomasal secretion in the

calf. Br. J. Nutr. 36:317.

Williams-Ashman, H.G., G.L. Coppoc, and G. Weber. 1972. Imbalance in ornithine metabolism in hepatomas of different growth rates as expressed in formation of putrescine, spermidine, and spermine. Cancer Res. 32:1924.

Wilson, R.H. and J. Leibholz. 1981. Digestion in the pig between 7 and 35 days of age. I. The performance of pigs given milk and soya-bean proteins. Br. J. Nutr. 45:301.

Winick, M. and A. Noble. 1965. Quantitative changes in DNA, RNA, and protein during prenatal and postnatal growth in the rat. Devel. Biol. 12:451.

Wright, N.A. 1982. The experimental analysis of changes in proliferative and morphological status in studies on the intestine. Scand. J. Gastro. 17 Suppl.(71-76):3.

Yamada, K., S. Bustamante, and O. Koldovsky. 1981. Dietary induced rapid increase of rat jejunal sucrase and lactase activity in all regions of the villus. FEBS Lett. 129:89.

Yang, P., S.B. Baylin, and G.D. Luk. 1984. Polyamines and intestinal growth: Absolute requirement for ODC activity in adaptation during lactation. Am. J. Physiol. 247:G553.

Yeh, K., F.W. Du, and P.R. Holt. 1986. Endogenous corticosterone rather than dietary sucrose as a modulator for intestinal sucrase activity in artificially reared rat pups. J. Nutr. 116:1334.

Yoshida, T., S. Numazawa, and Y. Kuroiwa. 1986. Induction of hepatic and renal ornithine decarboxylase by cobalt and other metal ions in rats. Biochem. J. 233:577.

Zamora, R.G. and T.L. Veum. 1978. Various levels of soybean meal as replacement for dried skim milk for artificially reared neonatal pigs. Nutr. Rep. Int. 18:495.