METHIONINE AND GLUCOSE TRANSPORT BY ISOLATED INTESTINAL BRUSH BORDER MEMBRANE VESICLES FROM PIGS AND LAMBS FED AN *ASPERGILLUS* PRODUCT

by

Insurk Jang

Dissertation submitted to the Faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Animal and Poultry Sciences

Approved:

K. E. Webb, Jr. Chairman

F. C. Gwazdauskas

A. F. Harper

J. H. Herbein, Jr.

E. T. Kornegay

M. D. Lindemann

October, 1993

Blacksburg, Virginia

METHIONINE AND GLUCOSE TRANSPORT BY ISOLATED INTESTINAL BRUSH BORDER MEMBRANE VESICLES FROM PIGS AND LAMBS FED AN *ASPERGILLUS* PRODUCT

by

Insurk Jang

Committee Chairman: K. E. Webb, Jr.

Animal and Poultry Sciences

(ABSTRACT)

This study was designed to determine whether feeding an *Aspergillus* product would influence growth or feed utilization and intestinal mucosal cell function as indicated by uptake of methionine and glucose by isolated intestinal brush border membrane vesicles (BBMV). In Experiment 1, 24 weanling pigs were paired by sex, BW, and litter and were allotted, within pairs, to either an 18% CP corn-soy diet (control) or the same diet supplemented (.15%) with an *Aspergillus* product. There were no differences ($P > .05$) in ADG, daily feed intake, or feed/gain between the two groups. In Experiment 2, 24 weanling wether lambs were paired by BW and were randomly assigned within pair to a 14% CP diet containing 61.1% cracked corn, 17.3% soybean meal, and 15% ground orchard grass hay (control) or the same diet fortified (.07%) with an *Aspergillus* product. There were no differences ($P > .05$) in ADG, daily feed intake, or feed/gain between the two groups. Enrichment of alkaline phosphatase in BBMV used in transport studies were 12.7-fold higher in pigs and 5.6-fold higher in lambs

over the original homogenate. Feeding the *Aspergillus* product did not change intestinal mucosal weight or total specific activities of alkaline phosphatase, sucrase, or Na^+/K^+ATPase in intestinal mucosal cells of pigs. Total uptake, Na^+-dependent uptake (including K_m and V_{max}), Na^+-independent uptake (including K_m and V_{max}), and diffusion of methionine and glucose were not changed by feeding the *Aspergillus* product. In lambs, jejunal mucosal weight decreased ($P < .08$) in response to the inclusion of the *Aspergillus* product, but the intestinal enzyme profile was not changed. The supplementation of the *Aspergillus* product tended to reduce the capacity for total uptake of glucose by jejunal and ileal BBMV largely due to decreased Na^+-dependent glucose uptake. The V_{max} for Na^+-dependent glucose uptake was greater ($P < .03$) in BBMV from lambs fed the control diet but K_m was similar for both groups. Total uptake, diffusion, and the kinetics (K_m and V_{max}) of Na^+-dependent and Na^+-independent uptakes of methionine were not influenced by diet. Sucrase activity of pigs was approximately 14% greater in the ileum than the jejunum. Alkaline phosphatase activity was approximately 500% greater in the jejunum than the ileum of lambs. Jejunal BBMV from lambs exhibited a greater capacity for total ($P < .04$), Na^+-dependent ($P < .03$), and diffusion ($P < .11$) uptake of glucose compared with ileal BBMV. The V_{max} for Na^+-dependent ($P < .002$) and Na^+-independent ($P < .006$) glucose uptakes were greater in jejunal BBMV than ileal BBMV from lambs. The relative importance of mode of uptake varied with substrate concentration for both glucose and methionine. Except for a decreased capacity for glucose uptake in lambs, feeding the *Aspergillus* product did not influence intestinal uptake of glucose or methionine.

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to my advisor, Dr. Kenneth Webb, Jr. for his support, encouragement, friendship, and invaluable assistance through the time it has taken me to complete for Ph.D. degree.

I would like to thank the members of my committee, Dr. F. C. Gwazdauskas, Dr. A. F. Harper, Dr. J. H. Herbein, Jr., Dr. E. T. Kornegay, and Dr. M. D. Lindemann for their caring comments and evaluations.

I wish to thank the fellow members of the protein laboratory during the research projects, especially Don Shaw, Kris Lee, Jamie Matthews, Shipping Wang, and Sharon Bowers.

Also, thanks go to my fellow graduate students, faculty, and staff, especially Han Swinkels who shared his knowledge and friendship during the past three years.

I also thank Sangho Kim and his family for their support and friendship during the past four years. In addition, I extend my appreciation to other Korean friends who made Blacksburg more than just a boring small university town.

A special thanks goes to my Korean advisor, Dr. K. K. Jung, who encouraged me to pursue a course of study that provided training in the fundamental aspects of biological sciences.

Finally, but most of all, I would like to express my sincere thanks my family, especially my parents, Jaehae Jang and Haesoon Jung, for their tolerance 3nd support through my extended education.

I would like to end the acknowledgments with this statement " getting a Ph.D. degree is not coming to a terminal station but just arriving at a starting station."

TABLE OF CONTENTS

Page

CHAPTER III

CHAPTER IV

LIST OF TABLES

CHAPTER III

APPENDIX

LIST OF FIGURES

CHAPTER III

Chapter I

Introduction

Probiotics (microbes and their metabolic by-products) are of interest as feed additives for both ruminants and non-ruminants to improve the efficiency of animal production for intensive management programs. This is partly because the use of antibiotics has a number of disadvantages such as the concern about possible tissue residues and the non-selective inhibitory effect on bacterial species in the gastrointestinal tract.

Alterations in the gastrointestinal microflora may have a marked effect on animal performance. Weaning animals at an early age is a common practice used to maximize animal productivity. However, early weaning can cause severe negative impacts including alteration of mucosal enzyme profiles and the morphology of absorptive cells, which leads to a subsequent reduction of intestinal transport capacity. The occurrence of some of these alterations has been linked to the absence of a normal gastrointestinal microflora. The administration of probiotics to weanling and young animals sometimes is effective in preventing or overcoming these problems. The animal feed industry is able to produce large quantities of probiotics economically. For these reasons, several probiotics currently are available commercially.

Among these, *Aspergillus* products are becoming widespread in their use due to their potential beneficial effects including increased nutrient digestibility, milk yield, and BW gains, although the responses are varied. The emphasis of most research has been on the effect of *Aspergillus* products on alteration of

nutrient digestibility or ruminal microbial fermentation that may lead to improvements in animal performance. However, the modes of action still are not clear. There is no direct evidence that *Aspergillus* products affect nutrient digestion and absorption in the small intestine. However, the potential for such modes of action should not be ignored.

The objective of the present study was to evaluate the possibility of altered intestinal transport in response to the feeding of *Aspergillus* products to pigs and lambs. Specific objectives included the study of glucose and methionine transport by brush border membrane vesicles prepared from enterocytes obtained from pigs and lambs that were fed a control diet or a diet fortified with an *Aspergillus* product. Glucose was chosen as a substrate because it is a major form of carbohydrate absorbed from the small intestine, especially in pigs. Methionine was selected as a test amino acid because of its importance as a dietary essential amino acid and as an amino acid that frequently may be limiting production.

Chapter II

Review of Literature

Amino Acid Transport in the Small Intestine

The cellular requirement of amino acids for tissue protein synthesis and metabolism is met by the cellular biosynthesis of some amino acids and the transport of other amino acids across the enterocyte of the small intestine. The small intestine is the primary site of amino absorption into mesenteric blood vessels in ruminant and non-ruminant animals. Transport of amino acids into blood vessels occurs by three continuous processes involving first the absorption of amino acids across the intestinal brush border membrane, then diffusion of the amino acids through the cytoplasm and finally liberation of these compounds through the basolateral membrane into mesenteric blood vessels (Argiles and Lopez-Soriano, 1990). For the most part, the early studies on the gastrointestinal transport of amino acids were associated with monogastric animals such as the pig, rat, rabbit, and guinea pig (Munck and Rasmussen, 1979; Sepeulveda and Smith, 1979; Munck, 1983) Relatively little information is known about transport of amino acid in ruminants. Several studies have demonstrated that peptides are readily absorbed and may play important roles in the overall supply of amino acids to tissues in ruminants (DiRienzo, 1990; Seal and Parker, 1992; Koeln et al., 1993). However, the significance of peptide transport still remains largely unexplored, especially with ruminants. Details of the current knowledge of amino

acid and peptide transport have been well reviewed (Matthews, 1975; Adibi and Kim, 1981; Webb, 1986; 1992).

The Significance of Site of Amino Acid Transport. The capability of amino acid transport in the small intestine is largely associated with the anatomical changes along the small intestine. It is generally agreed that transport activities vary with intestinal site. The middle to lower jejunum in monogastric animals such as the rat, guinea pig, and human has been identified as the primary site of amino acid absorption (Matthews and Laster, 1965; Schedl et al., 1968; Baker and George, 1971). In ruminants, however, the ileum is considered as the more active site for some amino acid transport. In sheep, it was shown that the ileum possessed the greatest absorptive capacity for threonine and valine and that the same amount of methionine was absorbed from the jejunum and ileum (Phillips et al., 1976). Presumably, the regional difference of amino acid transport may be due to varying affinities of the collective compliment of transporters, the capacities of transporters for individual amino acids, and the distribution of transporters along the intestine (Webb, 1992). According to Wilson and Webb (1990a), ileal brush border membrane of the bovine has a greater uptake capacity compared to jejunal brush border membrane. Additionally, they noted that methionine transporters had lower affinities and higher capacity for that amino acid than did lysine transporters in both the jejunum and the ileum. Transport studies that have investigated the pH dependence of amino acid transport have led to controversial results. Stingelin et al. (1986) observed that glutamate uptake was stimulated while lysine uptake was depressed at pH 4 compared to pH 5.5 or 7. But, leucine uptake was not influenced by the alteration of pH in ovine duodenal tissue. Wakeham and Webb (unpublished) observed that the uptake of methionine by bovine brush border

membrane vesicles (BBMV) was not influenced by changes in pH ranging from 5.0 to 8.0. Maenz et al. (1992) reported that the maximal transport of acidic amino acids, L-glutamic and D-aspartic acid, by brush border membrane of rabbits was represented by an optimal pH of 7.0. The range of pH from 7.2 to 8 appears optimal for intestinal proteases. In the ruminant, the contents of the small intestine usually reach this range in the last one-half to two-thirds of the intestine (Ben-Ghedalia et al., 1974). Thus, the luminal environment of the ileum in the ruminant may have more desirable conditions for maximal proleolytic activities and transport capacity for amino acids.

In comparison with amino transport of rat, hamster, and human intestine, several studies of intestinal amino acid transport with rabbits demonstrated that the distal ileum had the maximal rate of transport (Munck, 1985; Munck and Munck, 1992a). The significance related to intestinal region is still not completely understood. Conceivably, species difference may be an important factor in determining the predominant site of amino acid transport.

Amino Acid Transport Systems. The brush border membrane possesses transport systems including simple diffusion, carrier mediated facilitated diffusion, and energy dependent active transport. In simple diffusion, a type of passive transport, amino acids traverse the bilayer membrane unaided by transporters. In facilitated transport systems specific transporters called permeases aid in the transport of amino acids. In active transport systems, metabolic energy is consumed to shift amino acids against a concentration gradient. Crane et al.(1961) proposed the first explanation of Na^+-dependent active uphill transport of glucose by the flux of Na^+ gradient. This viable hypothesis was later extended to active transport of amino acids by Curran et al. (1967). In general, cells maintain a low

intracellular Na^+ concentration and a high intracellular K^+ concentration along with a net negative electrochemical potential inside (Darnell et al., 1990). This electrochemical potential gradient generated by the Na^+/K^+ pump provides free energy for active transport of amino acids. The overall process of this transport mechanism is that the Na^+/K^+ATPase located in the basolateral membrane of the enterocyte drives three Na^+ ions out of and two K^+ ions into the cytoplasm at the cost of one molecule of ATP (Fujiata et al., 1972). The two categories of carrier mediated transporter are saturable, so these systems have binding affinity constants (K_m) and maximum rates of transport (V_{max}). The active transport of peptides is proposed to be driven by cotransport with H^+ and an electrochemical gradient (Ganapathy and Leibach, 1985). The Na^+/H^+ exchanger in the brush border membrane is responsible for maintaining the proton gradient.

A number of distinct carrier mediated amino acid transport systems have been classified according to various categories of amino acids such as neutral, basic, and imino acids, as both Na^+-dependent and Na^+-independent pathways (Stevens et al., 1984), but the exact number is still unknown. The characterization of transport pathways based upon substrate preference is determined by measurement of kinetics, inhibitors, and competition analysis (Christensen, 1984). Size, charge, and configuration of amino acid side chains seem to be crucial factors involved in a particular transport system. Some amino acids share multiple carrier pathways (Christensen, 1984). Christensen (1984) suggested that 12 amino acid transport systems (A, ASC, L, Y+, Gly, N, b, L1, T, $X\text{-}_A$, $X\text{-}_G$ and $X\text{-}_{AG}$) occur in animal nonepithelial tissue. The A, ASC, L , Y^+ and $X\text{-}_{AG}$ systems have been identified in both epithelial and nonepithelial tissues. The remaining of the transport systems have been shown to be present in nonepithelial cells but not in

epithelial cells to date. Argiles and Lopez-Soriano (1990) described that at least four Na^+-dependent transport systems such as the neutral brush border (NBB), PHE, and imino X^-_{AG} carrier systems and two Na^+-independent transport systems including the L and Y^+ occur in the intestinal brush border membrane. The NBB system transports most neutral amino acids with some interaction of 2-aminonorborbonyl-2-carboxylic acid (BCH), which is a typical substrate for the L-system in other type membranes. This system does not share with MeAIB, so it is definitely a different pathway from the A system found in other cell types (Stevens et al., 1984). It is also different from the ASC system in that the NBB system is able to transport glycine and phenylalanine (Stevens et al., 1984). The PHE system primarily serves as the carrier mediated transport for methionine and phenylalanine. The imino carrier system is defined as a Na^+-dependent transporter of both aliphatic and cyclic imino acids (proline and hydroxyproline) and MeAIB (Munck and Munck, 1992b). This system is distinctly different from the A system, which is responsible for transport of MeAIB, alanine, and short chained polar amino acids (Stevens et al., 1984). The imino transporter was successfully labelled by Wright and Peerce (1984) using flurorescein and N-acetyl imidazole, which are attached near the proline binding site and near the binding site of Na^+. This suggests that the interaction of the imino transporter with Na^+ gives rise to the conformational alteration of the imino transporter. The X^-_{AG} Na^+-dependent system is responsible for transport of acidic amino acids including L-glutamic acid and D-aspartic acid (Malo, 1991). A salient feature of this transporter is that K^+ or H^+, in addition to Na^+ is also known to participate in counter transport (Corelli and Storelli, 1983; Berteloot, 1984).

In brush border membrane, at least two major Na^+-independent systems are known. The L system, one of two major Na^+-independent systems, preferentially transports neutral and lipophilic amino acids such as phenylalaline, leucine, and the 2-aminonorborbonyl-2-carboxylic acid (BCH; Stevens et al., 1984). This system is both pH and Na^+ insensitive (Oxender et al., 1977). The other Na^+-independent carrier system is Y^+, which is associated with transport of basic amino acids including lysine and arginine (Hopfer, 1987).

Transport of amino acids across the basolateral membrane is thought to occur via the Na^+-dependent A and ASC system and the Na^+-independent L system (Stevens et al., 1984). These systems may play a significant role in the supply of amino acids for maintenance and metabolism of enterocytes in some physiological circumstances (Argiles and Lopez-Soriano, 1990). The basolateral membrane is much more permeable to amino acids compared to brush border membrane, partly due to the leaky nature of this membrane. The A and ASC systems transport short chained, polar amino acids and three to four carbon neutral amino acids, respectively (Stevens et al., 1984). The L system appears to be the major transport pathway for neutral amino acids (Lash and Jones, 1984). However, none of intestinal transporters responsible for amino acid transport have been isolated and cloned so far.

The Relative Importance of the Pathways. The relative contribution of each mode of transport is highly correlated with the substrate present. Stevens et al. (1984) observed that Na^+-independent carrier mediated transport by the brush border membrane was dominant quantitatively at low concentrations of phenylalanine (< 1 mM). They also indicated that diffusion became the major contributor as substrate concentrate increased. Guerino and Baumrucker (1987)

observed that Na^+-independent transport made the greatest contribution to total uptake for both lysine and methionine when the substrate concentration was 1 mM in isolated ileal mucosa. Moe et al. (1987) observed that the relative contribution of active transport, facilitated diffusion, and simple diffusion accounted for 14.0, 37, and 49 % of total methionine uptake and 9.0, 53.0, and 38 % of total lysine uptake when the substrate concentration was .1 mM in bovine ileal BBMV. Wilson and Webb (1990a) reported that, at very low substrate concentrations (< 1.0 mM), facilitated diffusion was the greatest contributor to total uptake of lysine by BBMV. Results from that study also suggested that diffusion was the most predominant form of methionine transport throughout the range of substrates investigated. Malo (1991) reported on the relative contributions of transport systems for seven different amino acids (leucine, alanine, methionine, glutamine, aspartic acid, lysine, and proline) by human fetal intestine at the .05 mM substrate concentration. In their experiment, diffusion was the most important contributor to the total uptake of methionine, lysine, and proline but the predominant pathway of leucine, alanine, and aspartic acid transport was Na^+-dependent under these conditions. Wolffram et al. (1986) observed that the saturable transport systems of leucine were responsible for the greatest transport up to a concentration of 10 mM by brush border membrane from the mid-jejumum of sheep and pigs. Karasov et al. (1986) noted that the Na^+-dependent pathways for six amino acids including leucine, methionine, lysine, proline, MeAIB, and aspartic acid were the most prevalent when the relative portion of uptake was measured at .01 mM substrate concentration. The relative percentage of active transport, facilitated diffusion, and simple diffusion to total amino acid uptake is undoubtedly variable depending upon the initial amino acid concentration evaluated in the transport study.

However, the general conclusion that can be drawn is that active transport is quantitatively the most significant contributor when substrate concentration is low and diffusion makes progressively greater contributions to total uptake of amino acids as substrate concentrations increase (Webb, 1992). However, care should be taken when drawing conclusions regarding intestinal amino acid transport when uptake at only one substrate concentration is evaluated (Webb, 1986).

Competition and Interaction during Amino Acid Transport. The transport of an individual amino acid is not always independent from the transport of other amino acids. Frequently, amino acids share transporters, thus competition or interaction is inevitable when absorption of several amino acids is occurring at the same time. Silk et al. (1980) found that six amino acids (methionine, leucine, valine, phenyalanine, arginine, and isoleucine; in order of absorption) were preferentially transported when a mixture of 16 dietary amino acids was evaluated in human intestine. Phillips et al. (1976) reported that absorption of methionine was much greater from everted sacs of ovine intestine compared with valine and threonine. The proportional disappearance of essential amino acids was far greater than that of nonessential amino acids from the small intestine of sheep (Christiansen and Webb, 1990a). Similar results in humans, sheep, and cattle have been reported (Adibi and Gray, 1967; Phillips et al., 1979; Christiansen and Webb, 1990b). Moriyama (1986) observed that both lysine and phenylalanine in human fetal intestine appeared to be transported faster than alanine, leucine, taurine, and valine. Collectively, these results suggest that dietary essential amino acids have a tendency to be absorbed to a greater extent than dietary nonessential amino acids when comparison of absorption is based upon a percentage basis of total uptake

(Webb, 1992). In addition, methionine tends to be transported more extensively than any other amino acid (Webb, 1990).

Phillips et al. (1979) observed that methionine, when perfused in a mixture of methionine, valine, and threonine through isolated intestinal segments, acted as a strong inhibitor to the absorption of both valine and threonine. Karasov et al. (1986) reported that Na^+-dependent uptake of the basic amino acid lysine was totally hindered by the neutral amino acid leucine, and uptake of the neutral amino acid leucine was also partly inhibited by the basic amino acid lysine. Similar competitive inhibition is reported in numerous studies (Munck, 1980, 1983; Stevens et al., 1982). These findings imply that competitive inhibition of uptake happens even between amino acids that do not share the same transporter.

Generally, methionine seems to be a potent inhibitor of the uptake of other amino acids by bovine intestinal brush border membrane (Moe et al., 1987). Guerino and Baumrucker (1987) observed that the presence of up to 1 mM lysine, alpha-aminoisobutyric acid, or cycloleucine did not significantly influence the uptake of methionine by isolated bovine ileal mucosa (.1 mM). However, Karasov et al., (1986) indicated that the two neutral amino acids, leucine and methionine, were completely inhibited by each other. Paterson et al. (1981) reported that the interaction between the basic and neutral amino acids may result in the decrease in the transport of basic amino acids at the brush border membrane.

All interactions among amino acids are not negative. Phillips et al.(1979) noted that the absorption of threonine was accentuated by the presence of methionine at low concentrations of threonine. The effect of competitions and interactions on intestinal amino acids uptake is still obscure.

In conclusion, species differences exist in site of amino acid transport. The passage of amino acids across the brush border membrane is mediated by simple diffusion, facilitated diffusion (Na^+-independent; non-energy requiring), and active transport (Na^+-dependent; energy requiring). Different amino acid pathways in Na^+-dependent (NBB, PHE, X^-_{AG} and imino system) and Na^+-independent (L and Y^+) exist in brush border membrane. There is competition or interaction among amino acid during transport even by separate carrier systems.

General Glucose Transport in the Small Intestine

D-glucose serves as a source of energy and as a primary precursor for other carbon-containing compounds (Granner, 1990). Absorption of glucose by the small intestine is regarded as one of the important transport mechanisms to supply energy to all tissues of the body. Therefore, much effort has been made to investigate the transport mechanisms of glucose. Studies conducted over the past three decades give us a fairly clear understanding of the mechanisms involved in the D-glucose transport from the small intestine. Three distinct uptake phenomena, active transport, facilitated diffusion, and simple diffusion, take place in the small intestine. The principles of each component of transport were already described in amino acid transport. Thus, this review will be largely focused on Na^+-dependent, active transport and related topics.

Site of Glucose Absorption. The general thought regrading the intestinal site of D-glucose transport is that the entire small intestine is capable of Na^+-dependent, active transport (Malo, 1988). The middle and distal jejunum are recognized as the most active sites of D-glucose absorption. In humans, D-glucose

is absorbed maximally in the distal jejunum, followed by the proximal jejunum and distal ileum (Harig et al., 1989). This observation is consistent with the finding that very high glucose concentrations are observed in the proximal small intestine after a meal (Gray, 1981). Karasov et al. (1983) reported that active D-glucose transport by the proximal jejunum of the mouse is 700 % higher than that of the ileum. Vega et al. (1992) also indicated that the middle and proximal jejunum more actively absorbed D-glucose by carrier mediated transport compared with the distal ileum.

Glucose Transport Mechanisms. The Na^+/glucose symporter was first recognized by Crane and his colleagues in 1960. By coupling glucose to Na^+, glucose is actively transported across the intestinal brush border membrane. This uphill transport is known as secondary active transport, which belongs to a major class of membrane D-glucose transport. The presence of multiple Na^+/glucose cotransporters has been demonstrated in the intestinal brush border membrane in rats, guinea pigs, bovine and humans (Kaunitz and Wright, 1984; Brot-Laroche, 1986; Kwan et al., 1987; Malo, 1988). However, more recently, a major Na^+-dependent system (low affinity or high K_m) and a minor Na^+-dependent system (high affinity or low K_m) are referred to in glucose transport studies. Harig et al. (1989) reported that the jejunum of human intestine had a major and minor D-glucose transport system, while the ileum possessed only a major D-glucose transport system. Most of the studies on the kinetics of intestinal Na^+/glucose symporters have been conducted with intact mucosa (Schultz and Curran, 1970), isolated cells (Kimmich, 1981), or brush border membrane (Keljo et al., 1985; Hopfer, 1987) in various species.

Wright et al. (1991) described the range of K_m of D-glucose in brush border membrane as .1 to 1 mM in the presence of saturating Na^+ concentrations. Stevens et al. (1984) reviewed several studies, which indicated that the range of Km and Vmax was .1 to 3.7 mM and 5 to 36 nmoles/mg protein/min, respectively in rabbits under equilibrium exchanger or zero-trans experiments. Kaunitz and Wright (1983) reported that measurement of D-glucose uptake over the wider range revealed a major, low affinity system (K_m = .34 mM; V_{max} = 11 nmoles/mg protein/min) and a minor, high affinity system (K_m = .03 mM and V_{max} = 3 nmoles/mg protein/min). A study with human intestine (Malo, 1988) indicated the presence of a low affinity and high capacity (K_m=4.2 mM; V_{max}=30.9 nmol/mg protein/min) and high affinity, high capacity (K_m=.37 mM; V_{max}=8.3 nmol/mg protein/min) systems in the jejunum and a single active transporter (K_m=1.2 mM; V_{max}=4.9 nmol/mg protein/min) in the ileum. Another study with brush border membrane of pig intestine (Wolfran et al., 1986) showed that the K_m and V_{max} for D-glucose transport to be .69 mM and 23.9 nmol/mg protein/min, respectively.

Ruminants possess similar D-glucose transport to that of monogastric animals (Beechey et al., 1987; Buddington and Diamond, 1989). Kaunitz and Wright (1984) observed that the values of K_m and V_{max} for major D-glucose transport system in bovine jejunal tissue were about .20 mM and 5.3 nmol/mg protein/min, respectively. Shirazi-Beechey et al. (1991a) reported that the values of K_m and V_{max} of glucose transport in vesicles prepared from lamb were .05 mM and 9 nmol/ mg protein/min, respectively. Ruminants appear to have markedly lower K_m and V_{max} values compared with monogastric animals. In ruminants, most of the dietary carbohydrates are fermentated to volatile fatty acids

by microorganisms in the rumen, therefore, relatively small amounts of hexose pass through the small intestine (Fahey and Berger, 1988). This physiological phenomenon is associated with a significantly reduced expression of brush border membrane Na^+-glucose cotransporters and its activity (Shirazi-Beechey et al., 1991a). D-glucose transport in lambs and pigs was compared and it was observed that V_{max} was 10 times lower and K_m was five times lower in lamb brush border membrane (Wolffram et al., 1986). A similar trend of lower K_m and V_{max} of bovine intestine was observed in other studies (Scharrer et al., 1979; Stevens et al., 1984).

Carnivores, whose natural diet is protein rich, also exhibit the smaller K_m and V_{max} compared to species whose diet source is carbohydrate rich. Karasov et al. (1986) stated that herbivores and ominivores had a general tendency for higher intestinal glucose absorption rate (due to increased V_{max}) compared to carnivores. Buddington et al., (1991) reported that the maximal glucose transport rate of the intestine of cats was far less than that of mink which is an omnivorous species. However, Wolffram (1989) showed that the apparent kinetic parameters of the cat intestine resulted in a 4.4 fold higher V_{max} and a 4.3 fold smaller K_m compared with the respective values for rabbit intestine.

The progress of identification and characterization of the Na^+/glucose transporter has been difficult because less than 1 % of the membrane protein is Na^+/glucose cotransporter (Stevens et al., 1984). The first tentative identification of a 73 KDa protein using a phlorizin-analogue photoaffinity labeling of brush border membrane was revealed by Semenza et al. (1984). Later, Hediger et al., (1987) cloned, expressed and sequenced the rabbit Na^+/glucose cotransporter and

subsequently, Hediger et al. (1989) did the same with human Na^+/glucose transporter.

The transported glucose within the enterocyte passes out of the cell into the blood across the basolateral membrane by simple plus facilitated diffusion (Wright et al., 1980; Hopfer, 1987). The intestinal facilitated transporter is part of a family of glucose transporters that are also found in erythrocytes, liver, muscle, and fat cells (Carruthers 1990; Gould and Bell, 1990).

The relative contribution of each mode of transport is highly dependent on the concentration of substrate present. Stevens et al. (1984) reported that transport by the high affinity system is dominant at low glucose concentrations (<.5 mM), but the diffusive system is more important at high concentration (> 2 mM). Wolffram et al. (1986) observed that the saturable transport component predominated up to a 5 mM D-glucose concentration. However, above 5 mM, the diffusive system is more important.

In conclusion, D-glucose traverses the brush border membrane via simple diffusion, Na^+-independent transport, and Na^+/glucose symport. The most active sites for D-glucose transport are the middle and distal jejunum in monogastric animals. The presence of a major Na^+-dependent system (low affinity) and a minor Na^+-dependent system (high affinity) is known for D-glucose transport. The observed range of K_m in the major Na^+-dependent transport of D-glucose is .1 to 4.2 mM. However, species differences exist in the affinity of D-glucose transporters.

Adaptation of Intestinal Transport to Dietary Substrates

The mechanisms by which the small intestine adapts the absorption of sugars and amino acids have been of interest. Understanding the factors that influence the regulation of intestinal nutrient transport may enable us to not only manipulate these specific regulatory process, but also develop more efficient animal production systems. However, little is known about the adaptive responses (intestinal morphology, intestinal digestive enzymes, and intestinal sugar and amino acid transporters) in the gastrointestinal tract associated with dietary regulation such as alteration of carbohydrate, protein, fat, and inclusion of ionophores and antibiotics. Moreover, few instances of conditions that elicit regulation of intestinal transport with the supplementation of probiotics such as fungi, yeast, and microbial products have been reported. Yet, a more complete understanding of the intestinal transporters and transport mechanism involved with dietary factors and other feed additives is needed. Therefore, this portion of the literature review will largely focus on aspects of the comparison of bacteria with mammalian intestine in terms of adaptive nutrient transport, the adaptational effects of dietary factors (carbohydrates, proteins, and fats), or other means (antibiotics and probiotics) on intestinal sugar and amino acid transport.

Comparison of Bacteria with the Small Intestine in terms of Adaptive Nutrient Transport. Research with bacteria gives much information regarding adaptive membrane transport, as bacteria are unique organisms in that they cannot regulate their substrates (Ferraris and Diamond, 1989). Many bacterial enzymes and transporters are modulated by their environmental conditions such as the presence of substrates or their products, because the survival of bacteria in various

media is solely dependent on the capability of these unicellular organisms to scavenge nutrients. Thus it is apparent that most bacteria possess a variety of adaptive mechanisms. Similarly, the gastrointestinal mucosal cells, like bacteria, are constantly exposed to multiple luminal environments. Mammalian enterocytes must also be capable of adapting nutrient transport to various circumstances such as changes in luminal milieu, internal physiology, and pathophysiology. The change of luminal environmental conditions offers multiple opportunities for adaptation to occur. The mechanisms of nutrient absorption of the small intestine are very similar to those of bacteria (Matthews and Payne, 1975). The several nutrient transport systems including simple diffusion, facilitated carrier mediated diffusion and active transport, which are known to exist in bacteria, also exist in the mammalian small intestine. A detailed description of transport systems was presented previously. In fact, there is much similarity between the structure of prokaryotic and eukaryotic transporters, suggesting that these transporters reflect a uniform mechanism of action (Fredlander and Muecker, 1992). Henderson et al. (1992) reported that the primary sequence of the mammalian glucose transporters is homologous to the bacterial glucose transporters (22 to 33% homology). However, there are a few distinct characteristics of bacterial transport systems compared to that of the small intestine. In bacteria, the major driver ions of active transport are usually protons (H^+), while in the small intestinal cells, sodium ions (Na^+) are of major importance. The Na^+/K^+ATPase common to the basolateral membrane of enterocytes is not directly involved in proton pumping (Russell et al., 1990). However, Na^+-dependent, active transport has been found in E.coli (Chen et al., 1985), rumen bacteria (Strobel and Russell, 1991), and marine bacteria (Droniuk et al., 1987). Another difference is that bacteria can utilize a much

greater variety of substrates compared to animals. For example, many eukaryotic cells prefer glucose transport to other sugars, because glucose is the only useful energy source, but bacteria can utilize other hexoses as energy sources (Erni, 1989). Another striking feature related to regulation of intestinal transporters is the time required for adaptation. Adaptive bacterial transporters respond to altered substrate within minutes, while intestinal transporters exhibit slow and modest regulation (time lags of .5 to 4 h; Ferraris and Diamond, 1989).

Adaptation Due to Regulation of Enterocyte Morphology and Its Turnover.

There are several proposed mechanisms including alteration of intestinal morphology, digestive enzymes, and transporters as to how the small intestine can modulate its transport capability in response to dietary substrates. First, alteration of villus morphology and cell kinetics such as migration rate and cell turnover rate may influence intestinal nutrient transport capability by changing the villus absorptive area and enterocyte function (Ecknauer et al., 1982). Over a 2- to 3-d cycle, intestinal epithelium is continuously dividing and differentiating in crypts, migrating toward the upper villi, and finally being sloughed off at the villus tips (Argenzio, 1989). Therefore, dietary adaptation of intestinal transport may be associated with the crypt-villus morphology and cell kinetics. Several previous studies demonstrated that starvation or consuming protein-deficient diets resulted in a reduction (15 to 52%) of cell migration rate and this correspondingly results in a decrease of villus height and cell turnover rate (Cheeseman, 1986; Ogura et al., 1989). These observations clearly indicate adaptive responses because rapid cell migration and turnover rate not only decrease protein and energy supply in the cell, but also decrease transport capability of enterocytes.

Apparently, slow cell migration rate in pigs enhanced the functional capacity of the villus surface for amino acid transport and intestinal hydrolase activity because of a very mature villus population (Miller et al., 1986). Meddings et al. (1990) demonstrated that mature microvillus membranes showed the greatest glucose transport in rabbits. Another study using pigs showed that the supplementation of avoparcin, an antibiotic feed additive, markedly decreased the mucosal cell division rate in the crypts of duodenal tissue, suggesting that the inclusion of antibiotics may impact on intestinal nutrient transport (Parker, 1990). Mink fed a high carbohydrate diet with negligible protein had a longer but thinner small intestine compared with mink fed a high protein diet (Buddington et al., 1991). However, Ferraris et al. (1992a) observed that feeding varied dietary carbohydrate and protein levels reciprocally, while keeping protein intake at a maintenance level, to mice did not induce any difference in terms of villus migration rate, cell life time, crypt depth, or villus height. Mice fed different dietary amino acids and proteins did not exhibit any diet-dependent differences in intestinal morphology including intestinal length, wet weight per centimeter, or mucosal weight (Stein et al., 1987). Feeding of various fatty acids did not influence mucosal surface area or cell migration rate (Thomson et al., 1989).

Adaptation Due to Modulation of Intestinal Digestive Enzymes.

Another mode of adaptive responses of the intestine to dietary substrates is the modification of intestinal digestive enzyme activities (sucrase, maltase, aminopeptidase, etc). These altered enzyme activities may influence the overall capacity of the small intestine to digest carbohydrates and proteins and, subsequently, this may potentially affect intestinal sugar and amino acid transport (Keelan et al., 1990). It seems that modulation of digestive enzymes associated

with the intestinal brush border membrane is related to alteration of cell turnover rate because a rapid cell turnover rate may generate an immature cell that is not efficient in the expression of digestive enzymes. Thomson et al. (1987) reported that hypoglycemia in rats caused enhanced sucrase activity in the intestine which consequently led to increased glucose transport. Interestingly, three enzymes, sucrase, alkaline phophatase, and gamma-glutamyl transpeptidsase (GGP), located in the intestinal brush border membrane, increased their activities in response to increasing dietary carbohydrate levels. While the activities of alkaline phophatase and GGP were increased by increasing dietary protein levels, dietary sucrose enhanced sucrase activity (Ferraris et al., 1992a). Also, in monkeys fed high fat and cholesterol there was higher maximum capacity (V_{max}) for sucrase, alkaline phosphatase, and maltase (Dhaunsi et al., 1989). However, Shirazi-Beechey (1991b) observed that lactase and dipeptidylpeptidase were not influenced by dietary factors or modification of intestinal structure. On the other hand, the supplementation of probiotics (*lactobacillus*) or antibiotics (tylosin) to preweanling pig diets had a marked effect on the expression of sucrase, lactase, and tripeptidase activities but had no influence on dipeptidase activity (Collington et al., 1990). This mode of action of probiotics and antibiotics may be associated with the expression of mucosal cell and subsequently intestinal nutrient transport.

Adaptation Due to Alteration of Transporter Kinetics and Their Regulatory Signals. Another mechanism that may account for altered sugar or amino acid (peptide) transport in response to changing dietary substrates may be the alteration of intestinal nutrient transporters. The regulation and modulation of intestinal transporters by dietary substrates is one of the important mechanisms suggested here. Two marked features are observed when bacterial transporters are compared

with intestinal transporters in terms of their amplitude and adaptation activity; first, the ratio of transporter activity of control to that of adaptation (peak- to - base) is numerically small, less than 2-fold, while that of bacteria is usually several thousands fold (Ferraris and Diamond, 1989). Feeding a high carbohydrate diet to mice resulted in only a 2-fold increase of the maximal transporter activity compared with that of carbohydrate free diet (Diamond and Karasov, 1984). Second, adaptation of intestinal transporters is relatively slower (1 to 3 d) than that of bacterial transporters (< 1 min; Ferraris and Diamond, 1989). Intestinal nutrient transporters are regulated by a complicated combination of dietary factors including level of nutrient, inclusion of antibiotics, and ionophores. The most distinctly observed modulations of intestinal transporters in response to dietary adaptation are enhanced transporter number and enhanced transporter activity without alteration of mucosal mass or passive permeability. In fact, results from most studies suggest that alteration of intestinal sugar and amino acid transporters by dietary substrates may be generally caused by a change of transporter capacity (V_{max}), rather than a modulation of apparent carrier affinity (K_m; Fedorak, 1990). According to the results of a glucose-protectable phlorizin binding experiment, enhanced intestinal glucose transport capacity (V_{max}) in response to dietary carbohydrates was due to the increased number of transporters along the crypt-villus axis (Ferraris et al., 1992b). Feeding diets containing high carbohydrate levels to mice, rats, and fish results in a stimulated D-glucose active transport as a result of increased maximal velocity (V_{max}) compared to low carbohydrate diets (Diamond and Karasov, 1984; Cheeseman and Harley, 1991; Titus, 1991).

It seems that carbohydrate sources do not play a crucial role in the regulation of intestinal sugar transport. Vega et al. (1992) reported that the

inclusion of different carbohydrate sources, while maintaining an iso-carbohydrate level in suckling pig diets, did not modify carrier mediated sugar transport.

In the ruminant, most dietary carbohydrate is fermentated to VFA by rumen microorganisms, as a consequence, only negligible amounts of hexose pass into the small intestine under normal feeding conditions. Intestinal transporters of sugars in lambs showed relatively low detectable transporter affinity (K_m) and maximal velocity (V_{max}) compared with those of pigs (Wolffram et al., 1986). The infusion of 30 mM D-glucose into the proximal intestine of adult sheep gave rise to a 40- to 80-fold greater Na^+-dependent glucose transport when compared to the same age of sheep fed grasses (Shirazi-Beechey et al., 1991a). This study suggested that the signal for up-regulatation of intestinal glucose transport is mainly due to the presence of glucose substrates for the Na^+-dependent glucose transporter in the gut. Therefore, it is postulated that the intact glucose may impact the transcription or translation of the Na^+-dependent glucose gene expression as the enterocytes migrate up the villus (Shirazi-Beechey et al., 1991a). In addition, nearly all of the studies have focused on the dietary adaptation of intestinal brush border membrane without consideration of basolateral membranes. Cheeseman and Harley (1991) reported that adaptive response to a high carbohydrate diet was due to enhanced brush border membrane glucose transporters as well as basolateral glucose transporters. Generally, it is considered that basolateral transporters are controlled by plasma sugar concentration while brush border membrane transporters are regulated by luminal or intracelluar glucose concentration (Ferraris and Diamond, 1989; Cheeseman and Harley, 1991).

Adaptation of intestinal amino acid transport to level of dietary proteins and amino acids (or peptides) is far more complicated because of the presence of

separate transporter systems such as acidic amino acid, basic amino acid, neutral amino acid, imino acid, peptide, and multiple shared transporters (Ferraris and Diamond, 1989). Generally, intestinal transport for nonessential, acidic amino acids and imino acid has a tendency to show a similar pattern of regulation as glucose transport (i.e. up-regulation; Karasov et al., 1983). For instance, active transport of L-proline and aspartate in mice was stimulated by an increase in dietary protein (Stein et al., 1987; Karsov et al., 1983). However, essential, basic, and neutral amino acids display up and down regulatory transport pattern (Ferraris and Diamond, 1989). At low levels of dietary protein (0 to 18 %), amino acid transport was decreased with increasing dietary protein. In contrast, at high concentrations (> 18 %), amino acid transport was increased with increasing dietary protein or amino acids (Ferraris and Diamond, 1989).

What is the signal for regulation of intestinal amino acid transport in response to dietary substrates? What induces intestinal glucose transport is already known. However, not much is known about the regulation of intestinal amino acid transporters, partly due to the existence of multiple intestinal amino acid transporters. There appears to be some discrepancy between the inducer of intestinal transport and the transported substrate. In other words, a given amino acid may not provide a regulatory signal for its transport alone (Ferraris and Diamond, 1989). For example, aspartate and valine acted as the best inducers of the acidic amino acid and neutral amino acid transporters, respectively. Meanwhile, the acidic amino acid aspartate was considered as the best inducer of basic amino acid transport (Stein et al, 1987). It seems that whole proteins, peptides, and amino acids might play similar roles for the expression of intestinal amino acid transporters (Ferraris and Diamond, 1989). However, the

administration of the defined diets, containing amino acids or peptides, to rats markedly hindered the active transport of neutral amino acids compared to that of complex protein diets (Ohkohchi et al., 1990).

The addition of dietary fat has a different adaptational mechanism compared with dietary sugars and amino acids. The proposed mechanism of adaptive responses is that diets enriched with fat induce the alteration of intestinal brush border membrane lipid composition resulting in modulation of physical-chemical properties, membrane fluidity, and function (Keelan et al., 1989). As a consequence, these changes may give rise to alterations in membrane permeability as well as activity of intestinal transporters (Brasitus et al., 1989). However, the effect of dietary fat on up or down regulation of intestinal transporters of sugar and amino acids are still controversial. Diets enriched with unsaturated triglycerols showed higher V_{max} of Na^+-dependent nutrient transport when compared with that of saturated triglycerol. Monkeys fed high fat and cholesterol diets also had profound increases in active glucose transport but decreases in amino acid transport as a result of a change in V_{max} but not K_m (Dhaunsi et al., 1989). In contrast, another study observed that a decrease in glucose and galactose transport was associated with diets enriched in polyunsaturated fatty acids compared to diets enriched in saturated fatty acids (Thomson et al., 1987).

In addition to adaptation of intestinal sugar and amino acid transport in response to dietary sugar, protein, and fat, feed additives such as probiotics, antibiotics, and ionophores sometimes have a significant impact on intestinal mucosal cell turnover rate and activities of carbohydrate enzymes as mentioned earlier. Furthermore, it is already known that ionophores such as monensin and lasolocid are responsible for increased Na^+/K^+ATPase activity and Na^+-mediated

active transport at the cellular level of the small intestine (Spears, 1990). Information is available which suggests that avoparcin improves the net absorption of amino acids from the small intestine of sheep (Macgregor and Armstrong, 1984). Virginiamycin also has been shown to enhance uptake of free amino acids by isolated intestinal loops in pigs (Dierick et al., 1986). Further, the addition of antibiotics to a high concentrate diet fed to sheep caused the significant enhancement of intestinal glucose transport by increased number or activity of glucose receptors (Parker, 1990). Although there is some evidence that inclusion of probiotics as feed additives to animal diets may influence the expression of mucosal cell function, no study has been conducted to evaluate how probiotics may be able to modulate intestinal nutrient transport. One study with ruminal bacteria indicated that *Selenomonas ruminantium*, which is an important bacterium in the rumen, markedly increased transport of lactate in the presence of *Aspergillus* products. Ruminants, which are physiologically and anatomically different from other herbivores, also show adaptation capability to the alteration of dietary substrates and other means (probiotics and antibiotics), providing that these substrates can remain active in the lower gastrointestinal tract (Parker, 1990; Shirazi-Beechey, 1991a).

The Significance of Intestinal Site and Species on Adaptation. The ability of the gut to adaptively modulate rates of sugar and amino acid transport is related to the site of the small intestine. Several studies with increased dietary carbohydrate, protein, and fat or inclusion of probiotics showed that the proximal intestine has a greater ability than the distal intestine to change intestinal digestive enzyme expression and transporters (Karsov et al., 1983; Dhaunsi et al., 1989; Parker, 1990; Buddington et al.,1991; Ferraris and Diamond, 1992).

Species may vary in their ability to adapt their intestinal transport capabilities. Herbivores and omnivores that habitually consume high carbohydrate or mixed diets, can modulate intestinal sugar and amino acid transport. In contrast, carnivorous animals accustomed to consuming a high protein diet lack the adaptive capability for intestinal sugar transport (Buddington and Diamond., 1989). However, these species generally are able to regulate intestinal amino acid transport.

In conclusion, the phenomenon of adaptation of intestinal glucose and amino acid transport is mediated by a number of factors including dietary substrate (level of carbohydrates, fats, and protein) or other means (probiotics and antibiotics). The several plausible mechanisms by which the small intestine may adapt are 1) the regulation of enterocyte morphology and turnover, 2) the modulation of intestinal digestive enzymes, and 3) the alteration of enterocyte transporter kinetics. It seems that these proposed mechanisms are closely related to one another for the expression of adaptation when the small intestine is exposed to various environments. Strong evidence has emerged which suggests that one of the most important modes of action of adaptive intestinal sugar and amino acid transport suggested is via the alteration of transporters by dietary substrates. This regulation of transporters is generally caused by a change of transporter capacity (V_{max}) rather than a alteration of transporter affinity (K_m).

Influence of Dietary Addition of Aspergillus Products on Nutrient Utilization and Animal Performance

Using microbes and their metabolic by-products as feed additives to enhance nutrient utilization by manipulation of the gastrointestinal environment has been common for several decades. Because concerns over possible antibiotics in animal products has led to the increased pressure to reduce antibiotic use, the development of alternatives including microbe, yeast, and fungal culture products (probiotics) is accelerating (Parker, 1990). Currently, feeding of fungal (*Aspergillus oryzae*) and yeast (*Sacharmyces cerevisiae*) fermentation products is becoming widespread. However, sometimes the beneficial effects obtained regarding animal performance and nutrient utilization from these probiotics are equivocal. *Aspergillus* products may play an important role in the degradation of cellulose, hemicellulose, protein, and starch because these products contain substantial amounts of cellulase, proteases, amylase, and lipases. Also, these microorganisms and their fermentation products contain B vitamins, minerals, and possibly unidentified growth factors (Boing, 1983; Mountfort, 1987; Kung, et al., 1992). Most researchers place emphasis on the effects of *Aspergillus* products on the altered nutrient digestibility in the rumen and altered rumen microbial fermentation. Direct evidence is not available to suggest that these products have an impact on the digestion and absorption of nutrients in the small intestine. However, the potential mode of action of *Aspergillus* products on nutrient digestion and absorption in the small intestine should not be overlooked. Additionally, fungal cultures are rarely investigated as dietary sources for pigs.

This literature review will largely be focused on the modes of action of *Aspergillus* products on nutrient digestion and microbial fermentation in ruminants and their effect of animal performance.

Modes of Action of *Aspergillus* Products. The purpose of fungal and yeast cultures for ruminants is to stimulate the increase of beneficial microorganisms naturally present in the gastrointestinal tract rather than supplying live organisms and, subsequently, to alter nutrient digestion and ruminal fermentation. (Haresign and Ewing, 1989). The mode of action of these products is still unclear, although fungi or yeast fermentation products appear to be effective in ruminants.

One of the mechanisms suggested is that fungal or yeast cultures stabilize rumen pH by increased lactate uptake by ruminal microorganisms. The study using the rumen simulation technique demonstrated that the inclusion of *Aspergillus* products to a basal diet prevented transient drops in rumen pH (Frumholtz et al., 1989). Lactate concentration tends to be associated with ruminal pH (Allison, 1989). Nisbet and Martin (1990) noted that *Selenomonas ruminatium*, which is a major cellulolytic bacterium in the rumen, exhibited a markedly enhanced lactate uptake in the presence of *Aspergillus* products. Williams et al.(1990) reported that the addition of yeast to high concentrate diets prevented a decline of ruminal pH due to a reduction of lactate accumulation. This ability to promote a stabilized pH may play an important role in the proliferation of cellulolytic bacteria, since these microorganisms are highly sensitive to fluctuations of rumen pH (Frumholtz et al., 1989). The enhanced number of cellulolytic or total viable bacteria in response to *Aspergillus* products was reported in the rumen of sheep and calves (Weidmeier et al., 1987; Beharka et al., 1991) and in rumen simulation technique (Newbold et al.,

1991; Frumholtz et al., 1989). Others, however, failed to observe increases in pH in response to *Aspergillus* products (Martin and Russell, 1986; Newbold et al., 1991). Another plausible explanation for the proliferation of cellulolytic bacteria is that *Aspergillus* products containing B vitamins, minerals, and unidentified growth factors may provide some direct stimulatory factors (Bryant, 1973; Wiesmeier et al., 1987).

Increased numbers of cellulolytic bacteria and their activities seem to be related to the improvement of dry matter, organic matter, and fiber digestibility. Wieismeier et al., (1987) demonstrated an improved dry matter and hemicellulose digestion when 50 % concentrate diets were supplemented with *Aspergillus* products in cows. Similar patterns of increased digestibilities with the supplementation of fungi or yeast to moderate to high concentrate diets were observed in ruminal or total tract dry matter (Williams et al., 1990; Caton et al., 1993), organic matter (Van Horn et al., 1984; Caton et al., 1993), and fiber such as NDF, ADF, and hemicellulose (Judkins and Stobart, 1988; Gomez-Alarcon et al., 1990, 1991; Newbold et al., 1991). In contrast, other studies failed to demonstrate the alteration of fiber digestion in response to *Aspergillus* products (Firkins et al., 1990; Denigan et al., 1992). Oellermann et al. (1990) reported that digestibilities of NDF and ADF were not affected by the addition of *Aspergillus* products in dairy cows fed high amounts of straw. One explanation of discrepancies among experiments may be that fiber sources, fiber types, and relative percent of concentrate in the diet are important factors to determine the effect of *Aspergillus* products on fiber digestion (Van Horn et al., 1984; Gomez-Alarcon et al., 1990).

The fungi or yeasts may be effective for ruminants fed high concentrate diets where often there is a depression in cellulose digestion. Feeding high

concentrate diets containing substantial amounts of starch and sugars has been adopted to improve the energy density of diets and, subsequently, increase performance of the animal. However, this feeding regimen causes negative associative effects such as decreased digestion of structural carbohydrate in grain and forage (Fahey and Berger, 1988) Evidently, negative results are associated with alteration of the cellulolytic bacteria habitat in the rumen ecosystem (Jahn et al., 1973). Thus, the inclusion of fungi or yeast products containing cellulolytic activity may improve structural carbohydrate digestion. *Aspergillus* products are capable of partial depolymerization of structural cellulose, although enzymes produced by fungi do not depolymerize cellulose completely to glucose (Boing, 1983). Furthermore, these fungi or yeast products can aid digestion of nonstructural carbohydrates such as starch and pectin because of their secretion of amylase (Ustyuzhanina et al., 1984). Williams et al. (1990) reported that diets fortified with a yeast (*Saccharomtces cerevisiae*) gave rise to a significant reduction of hexose-unit oligosaccharide (3-fold) in ruminal fluid of steers.

Aspergillus products also contain proteolytic activity to assist digestion of ingested protein (Boing et al., 1983). Several studies indicated that fungi or yeast cultures promoted ruminal or total tract apparent digestibilities of crude protein in vivo and in vitro (Arambel et al., 1987; Wiedmeiser et al., 1987; Gomez-Alarcon et al., 1990). However, the diets containing high fiber (chopped straw) did not show an increase in crude protein digestibility (Oellermann et al., 1990). Caton et al. (1993) observed that total, essential, and nonessential amino acid flow rate was markedly increased by the addition of aspergillus products in dairy cows.

Fungi and yeast products have been reported to alter the stoichiometry of rumen fermentation. Martin and Russell (1986) observed that feeding *Aspergillus*

products increased total VFA, all of the individual VFA, and ammonia concentration. Arambel et al. (1987) showed that *Aspergillus* products in vitro increased the ruminal ammonia and total and branched-chain VFA concentrations owing to deamination and decarboxylation of branched chain amino acids (Allison and Bryant, 1963). However, results from several studies where low to moderate levels of concentrate were fed, no change of total VFA, ammonia, or individual VFA was observed (Fondevila et al., 1990; Oellermann et al., 1990; Newbold et al., 1991; Caton et al., 1993). There is a general lack of agreement regarding the effect of *Aspergillus* products on rumen VFA and ammonia production. It seems that alteration of fiber digestion is responsible for the beneficial impact of fungi or yeast products, rather than chemical changes of rumen fermentation.

Interestingly, in contrast to control cows, the duodenum of cows fed *Aspergillus* products possessed feed particles containing a dense population of fungi similar to normal rumen fungi under microscopic examination (Prange et al., 1979). This observation suggests that *Aspergillus* products may have an impact on the degradation of fiber components in the intestine as well as in the rumen.

The Effect of Aspergillus Products on Animal Performance. Improvement in nutrient digestion and rumen fermentation may be associated with the improvement of animal performance. Slippers et al. (1988) reported that steers fed *Aspergillus* products exhibited increased growth rates and feed efficiencies. Furthermore, higher fat-corrected milk or milk production in dairy cows fed *Aspergillus* products have been observed (Gomez-Alarcon et al., 1991). These responses varied according to the type of diet and stage of lactation. The greatest responses were achieved when *Aspergillus* products were used in early lactation cows and in cows fed high concentrate diets (Gomez-Alarcon et al., 1991).

However, results from other studies will not confirm a response of improved performance with the feeding of *Aspergillus* products. Denigan et al. (1992) noted that the supplementation of *Aspergillus* products to 60% concentrate diets in dairy cows did not improve feed intake, feed efficiency, rectal temperature, or milk yield. Other studies with lactating cows failed to observe the improvement of milk yield and fat corrected milk production (Kellems et al., 1990; Higginbotham et al., 1993). Herring and Hallford (1991) reported that, although feed intake, performance and serum hormones (growth hormone, insulin, and prolactin) were not altered, lambs fed a moderate concentrate diet fortified with an *Aspergillus* product tended to have improved backfat and percent of wholesale cuts in carcass.

Aspergillus Products for Pigs. Fungi or yeast cultures are rarely investigated in swine production, although these products are already known to contain substantial amounts of digestive enzymes, B vitamins, minerals, and possibly unknown growth factors. Growing pigs fed a diet supplemented with a yeast single cell protein had improved nitrogen digestibility and retention (Tegbe and Zimmerman, 1977). Neonatal pigs fed heated whole soybeans fermented with *Aspergillus oryzae* did not show any difference in average daily gain, feed efficiency, biological value, and crude protein digestibility compared with pigs fed heated whole soybeans (Zamora and Veum, 1988).

In conclusion, the results suggest that *Aspergillus* products may have impact on the number of cellulolytic bacteria in rumen, the digestibility of fiber and rumen fermentation, and subsequently the increase in animal performance under moderate or high concentrate diets, although the responses are variable. In addition, much more research is necessary to determine the efficacy of *Aspergillus* products on the rumen and the lower gut and for the inclusion in the diets of pigs.

Chapter III

Methionine and Glucose Transport by Isolated Intestinal Brush Border Membrane Vesicles from Pigs and Lambs Fed an *Aspergillus* Product

ABSTRACT

This study was designed to determine whether feeding an *Aspergillus* product would influence growth or feed utilization and intestinal mucosal cell function as indicated by uptake of methionine and glucose by isolated intestinal brush border membrane vesicles (BBMV). In Experiment 1, 24 weanling pigs were paired by sex, BW, and litter and were allotted, within pairs, to either an 18% CP corn-soy diet (control) or the same diet supplemented (.15%) with an *Aspergillus* product. There were no differences ($P > .05$) in ADG, daily feed intake, or feed/gain between the two groups. In Experiment 2, 24 weanling wether lambs were paired by BW and were randomly assigned within pair to a 14% CP diet containing 61.1% cracked corn, 17.3% soybean meal, and 15% ground orchard grass hay (control) or the same diet fortified (.07%) with an *Aspergillus* product. There were no differences ($P > .05$) in ADG, daily feed intake, or feed/gain between the two groups. Enrichments of alkaline phosphatase in BBMV used in transport studies were 12.7-fold higher in pigs and 5.6-fold higher in lambs over the original homogenate. Feeding the *Aspergillus* product did not change intestinal mucosal weight or total specific activities of alkaline phosphatase, sucrase, or Na^+/K^+ATPase in intestinal mucosal cells of pigs. Total uptake, Na^+-dependent uptake (including K_m and V_{max}), Na^+-independent uptake (including

K_m and V_{max}), and diffusion of methionine and glucose were not changed by feeding the *Aspergillus* product. In lambs, jejunal mucosal weight decreased ($P < .08$) in response to the inclusion of the *Aspergillus* product, but the intestinal enzyme profile was not changed. The supplementation of the *Aspergillus* product tended to reduce the capacity for total uptake of glucose by jejunal and ileal BBMV largely due to decreased Na^+-dependent glucose uptake. The V_{max} for Na^+-dependent glucose uptake was greater ($P < .03$) in BBMV from lambs fed the control diet but K_m was similar for both groups. Total uptake, diffusion, and the kinetics (K_m and V_{max}) of Na^+-dependent and Na^+-independent uptakes of methionine were not influenced by diet. Sucrase activity of pigs was approximately 14% greater in the ileum than the jejunum. Alkaline phosphatase activity was approximately 500% greater in the jejunum than the ileum of lambs. Jejunal BBMV from lambs exhibited a greater capacity for total ($P < .04$), Na^+-dependent ($P < .03$), and diffusion ($P < .11$) uptake of glucose compared with ileal BBMV. The V_{max} for Na^+-dependent ($P < .002$) and Na^+-independent ($P < .006$) glucose uptakes were greater in jejunal BBMV than ileal BBMV from lambs. The relative importance of mode of uptake varied with substrate concentration for both glucose and methionine. Except for a decreased capacity for glucose uptake in lambs, feeding the *Aspergillus* product did not influence intestinal uptake of glucose or methionine.

Introduction

Much is known about the mechanisms of absorption and transport of amino acids and glucose by the small intestine. Yet a more complete understanding of how dietary factors may regulate intestinal transporters and transport mechanisms is needed. Research with bacteria revealed how the bacterial cell can modulate its transport capability in response to dietary substrates (Fearris and Diamond, 1989). The mechanisms of nutrient absorption in the small intestine and the primary sequence of transporters are very similar to those of bacteria (Matthews and Payne, 1975; Henderson et al., 1992). The mucosal cells of the small intestine are actively responsive to physiological, nutritional, and environmental stimuli (Fedorak, 1990). The mammalian enterocytes, like bacteria, are constantly exposed to multiple luminal environments by the alteration of diet composition (protein, carbohydrate, and fat) and dietary supplementation (antibiotics, ionophores, and probiotics). Subsequently, the altered luminal conditions offer multiple opportunities for adaptation to occur. There are several ways the small intestine may adapt including the alteration of intestinal morphology (mucosal cell turnover, mass of mucosal cells, etc.), the change of digestive enzymes (sucrase, maltase, aminopeptidase, etc), and the modulation of membrane transporters (K_m or V_{max}; Keelan et al., 1990). Evidence has emerged that indicates that one of the most important modes of adaptive intestinal glucose and amino acid transport is via alteration of transport. In fact, the alteration of intestinal transport proteins by dietary substrates may be generally caused by a change of transporter capacity (V_{max}), rather than a modulation of apparent transport protein affinity (K_m; Fedorak, 1989; Argiles and Lopez-Soriano, 1990). Results from studies with mice,

fish, humans, monkeys, and sheep indicated that elevated levels of glucose or amino acids in the diet result in a stimulated glucose or amino acid transport, respectively, by the intestine as a result of increased V_{max} (Diamond and Karasov, 1984; Shirazi-Beechey et al., 1991a; Titus et al., 1991).

It is well documented that ionophores such as monensin and lasolocid, are responsible for increased Na^+/K^+ATPase activity and Na^+-dependent, active transport at the cellular level of the small intestine (Spears, 1990). The inclusion of certain antibiotics in pig or sheep diets has been shown to cause significant enhancement of intestinal glucose and amino acid absorption or the expression of intestinal mucosal cell function (Dierick et al., 1986; Collington et al., 1990; Parker, 1990). There is limited evidence that supplementation with probiotics enhances intestinal digestive enzymes including lactase and sucrase (Collington et al., 1990).

Among probiotics, *Aspergillus* products have been used as feed supplements for ruminants due to their potentially beneficial effects such as improved crude protein and fiber digestibility, increased milk yields and improved BW gains with moderate to high concentrate diets. However, the responses have been variable. No studies have been reported where *Aspergillus* products have been included in the diets of pigs. Most research has focused on studying the effect of *Aspergillus* products on nutrient digestibility in the rumen and altered rumen microbial fermentation. Results from a study with *Selenomonus ruminatium* indicated that nutrient transport (lactate) is markedly stimulated in the presence of *Apergillus* products (Nisbet and Martin, 1990). If *Aspergillus* products influence transport of nutrients across bacterial membranes, they may affect mammalian intestinal brush border membranes similarly.

Therefore, the present study was conducted to determine 1) growth and feed utilization of pigs and lambs fed an *Aspergillus* product and 2) methionine and glucose transport by isolated intestinal brush border membrane vesicles from pigs and lambs fed an *Aspergillus* product.

Materials and Methods

Animals and Feeding

Experiment 1. Twenty four pigs were weaned at 4 wk of age and paired by sex, BW, and litter. Each pig was then placed in a pen in a room equipped with temperature and ventilation control. During a 7-d adjustment period following weaning, the pigs were fed the 20% crude protein, soy-corn-whey diet shown in Table 1. Immediately after this period, one pig from each pair was chosen at random to be fed an 18% crude protein soy-corn diet (control) or the same diet fortified with .15% *Aspergillus* product (*Aspergillus,* Table 1). The *Aspergillus* product was Amaferm and was supplied by BioZyme Inc, St. Josepth, Mo. The pigs were weighed initially (average 6.75 kg) and then at weekly intervals for 5 wk for the calculation of ADG. Water and feed were offered ad libitum and feed consumption was recorded weekly to determine feed intake and efficiency of feed utilization. The feeding trial was continued for 35 d. Vitamins and trace minerals were added to diet at the level of NRC (1988) requirements.

Experiment 2. Twenty-four weanling, wether lambs were paired based upon BW and breeding. The lambs were housed individually in pens with elevated, expanded metal floors in a room equipped with temperature and ventilation control. One lamb from each pen was randomly chosen to be fed either

Table 1. Ingredient composition of diets fed to weanling pigs (as fed basis)

Items	Diets		
	20% CP[a]	Control[b]	Aspergillus[b]
	---------- % ----------		
Ground corn	51.75	70.25	70.10
Soybean meal	30.95	27.33	27.33
Dried whey	15.00	-----	-----
Limestone	.59	.52	.52
Defluorinated phosphate	1.06	1.25	1.25
Salt	.30	.30	.30
Trace mineral premix[c]	.10	.10	.10
Vitamin premix with Se[d]	.25	.25	.25
Aspergillus product[e]	-----	-----	.15

[a]Calculated to contain 20% CP, .8% Ca, and .65% P.
[b]Calculated to contain 18% CP, .7 % Ca, and .6% P.
[c]Supplied per kg of diet: 150.1 mg Zn, 175.3 mg Fe, 60.0 mg Mn, 17.4 mg Cu and 2.0 mg I.
[d]Supplied per kg of diet: 1.1 mg vitamin K_3 (MPB), 4.4 mg riboflavin, 22 mg niacin, 441 mg choline, 22 mg pantothenic acid,02 mg vitamin B_{12}, .4 mg biotin, 2.0 mg folic acid, 4,409 IU vitamin A, 441 IU vitamin D_3, 11 IU vitamin E and 31 mg selenium.
[e]Amaferm, BioZyme Inc., St. Joseph, Mo

a 14% crude protein diet (control; 12 lambs) or the same diet supplemented with (.07 %) *Aspergillus* product (*Aspergillus*; 12 lambs). Vitamins were provided by intramuscular injection. The detailed composition of diets is presented in Table 2. The lambs were weighed initially (average 21.9 kg) and at weekly intervals to determine ADG. Water and feed were offered ad libitum and feed consumption was recorded twice a day for the calculation of feed intake and efficiency of feed utilization. The feeding period was 91 d.

Tissue Sampling

At the end of the feeding period, six pairs of pigs and six pairs of lambs were randomly selected and sacrificed for collection of intestinal mucosa. Pigs and lambs were anesthetized by intravenous injection of sodium pentobarbitol (32.5mg/kg BW) and then were euthanized by exsanguination. The entire small intestine was rapidly removed and divided in half. The proximal half was designated as the jejunum and the distal half as the ileum. Each section was segmented into approximately 40 cm lengths and cut open longitudinally along the mesenteric border. The opened segments were rinsed in three successive baths containing mannitol buffer composed of 30 mM mannitol and 12 mM tris base (pH; 7.4). The segments then were incubated in a hyaluronidase buffer containing 1 mg hyaluronidase/mL, 1 mg bovine serum albumin/mL, 120 mM NaCl, 20 mM tris base, 1 mM $MgCl_2$ and 3 mM K_2HPO_4 (pH;7.4) for 20 min at 37°C. Immediately after incubation, the intestinal segments were placed in an aluminium pan that was seated on a bed of ice. The mucosal surface was removed by gentle scraping with a glass slide. Residual hyaluronidase buffer was removed from harvested mucosal scrapings by resuspending twice in equal volumes of mannitol

Table 2. Ingredient composition of diets fed to weanling lambs, as fed basis.

Items	Diets[a]	
	Control	Aspergillus
	---------- % ----------	
Orchardgrass hay	15.00	15.00
Cracked corn	61.12	61.05
Soybean meal	17.33	17.33
Molasses	5.00	5.00
Limestone	1.12	1.12
Trace mineral salt[b]	.50	.50
Vitamins plus Se[c]	++	++
Aspergillus product[d]	--	.07

[a]Calculated to contain 14% crude protein, .50% Ca, and .35% P.
[b]NaCl, 96 to 98.5%; Zn ≥ .35%; Fe, ≥ 3.4%; Mn, ≥ .20%; Cu, ≥ .033%; I, ≥ .0007%; Co, ≥ .005%.
[c]At the start of the experiment, all lambs received an intramuscular injection of vitamin A, 500,000 IU; vitamin D, 75,000 IU; vitamin E, 5 IU; and selenium 55 $ug.kg^{-1}$.
[d]Amaferm, BioZyme Inc., St. Joseph, Mo.

buffer followed each time by centrifugation at 4,500 x *g* at 4°C for 12 min. The harvested intestinal mucosa was divided into approximately 40 g aliquots and each aliquot was placed in a sealed plastic bag and frozen in liquid nitrogen. The tissue was then stored at - 70°C.

Preparation of Brush border Membrane Vesicles

Brush border membrane vesicles (BBMV) were prepared by a modification of the procedure presented by Wilson and Webb (1990b). All steps of the procedure were performed in such a manner as to maintain the tissue membranes at approximately 4°C. Approximately 40 g of intestinal mucosa were suspended in mannitol-succinate buffer (1 g tissue / 8 mL buffer) containing 5 mM $MgCl_2$, 150 mM mannitol, 10 mM tris base, 30 mM succinate, 5 mM K_2HPO_4 and .1 mM $MnCl_2$ (pH;7.4) and homogenized using a polytron (PT 10/35 polytron, Brinkman Instrument, Wesburg NY) equipped with a 20 cm diameter probe, for 20 s at 13,200 rpm. An aliquot of the homogenate was sampled for the determination of protein and marker enzymes. The homogenate then was incubated with mannitol succinate buffer for 30 min while gently stirring to allow Mg^{++} precipitation of internal membranes such as endoplasmic reticulum, lysosomes, and mitochondria (Kessler et al., 1978). Following incubation, the homogenate (H) was centrifuged at 8,700 x *g* for 12 min at 4°C. The resulting supernant (S1) was centrifuged at 31,000 x *g* for 15 min. The resulting pellet (P2a) was suspended in a mannitol-succinate buffer and centrifuged at 31,000 x *g* for 15 min. The resultant pellet (P2b) was resuspended in a mannitol-succinate buffer with 12 strokes of a teflon-glass homogenizer (Kontes Scientific Glassware, Vineland, NJ) and continuously incubated for 30 min with gentle stirring. The suspension was centrifuged at 8,700

x *g* for 12 min. The supernant (S3) then was centrifuged at 31,000 x *g* for 15 min. The resulting pellet (P4) was resuspended in a mannitol-transport buffer containing 305 mM mannitol, 20 mM HEPES, and 2 mM $MgCl_2$ (pH; 7.4) with 12 strokes of a telfon-glass homogenizer to a final protein concentration of 2 to 3 mg/mL. The suspension was carefully applied on the top of a density gradient made of 3.5 mL of 27 % sucrose solution and 3.5 mL of 31% sucrose solution. Sucrose solutions contained 27% or 31% dried sucrose in 4 mM $MgCl_2$ and 4 mM HEPES. Care was taken to avoid mixing of the three layers in the centrifuge tube. The gradient was centrifuged (Model L5-75 B with SW- 40 rotor, Beckman Instrument, Palo Alto, CA) at 105,000 x *g* for 90 min. The resultant two bands and pellet were harvested by aspirating the membrane band with the underlying sucrose layer. Samples were taken for the assay of protein and marker enzymes and the remainder of each band was put into a cryovial (Nunc cryotubes, Vingard International Inc., Neptune, NJ), frozen in liquid nitrogen, and stored at -70°C until used for transport experiments.

Characterization of Brush Border Membrane Vesicles

For characterization experiments, all enzymes described below and protein were determined for all fractions (H, P1, S1, P2a, S2a, P2b, S2b, P3, S3, P4, and S4) and bands (B I and B II) created during the isolation process. To assess the isolation procedure of BBMV, protein and total enzyme activities of organelles in all fractions were measured and percent recovery was calculated at each differentiation step. The degree of purification of BBMV isolated was determined by the enrichment of the specific activity of the brush border marker enzyme, alkaline phosphatase (pigs and lambs) and sucrase (only pigs) with respect to the

original homogenate. The degree of contamination from other cellular organelles was evaluated by the enrichment of the marker enzymes Na^+/K^+ATPase for basolateral membrane, acid phosphatase for lysosomes, lactate dehydrogenase for cytosol, NADP-cytochrome c reductase for microsomes, and cytochrome c oxidase for mitochondria. Both jejunal and ileal tissues from pigs and lambs were used for characterization experiments. Subsequently, when BBMV were prepared for transport studies, only protein, alkaline phosphatase, sucrase (for pigs only) and Na^+/K^+ATPase were assayed in the original homogenate and the bands harvested from the density gradient. Brush border membrane vesicles were prepared from six pairs of pigs and six pairs of lambs for transport assays.

Alkaline phosphatase (E.C.3.1.3.1), the marker enzyme for brush border membrane, was assayed using a Sigma Diagnostic Assay Kit (Procedure No. 245) with a computer controlled vertical photometer (Titertek Multiskan MCC/340, Flow Laboratories, McLean, VA). Specific activity was expressed per milligram of protein.

Sucrase (EC 3.2.1.26), the marker enzyme of brush border membrane for monogastric animals, was determined by a modification of the procedure of Dahlqvist (1968). The end product, glucose, was measured by spectrophotometric assay.

Ouabain sensitive Na^+/K^+ATPase (E.C.3.6.1.3), the marker enzyme of basolateral membrane, was detected by modification of the method of Jorgenson (1975). The end product, liberated inorganic phosphate from hydrolysis of ATP, was measured colorimetrically (Ebil and Lands, 1969). The activity of Na^+/K^+ATPase was obtained by subtracting ouabain sensitive ATPase activity from total ATPase activity which was measured in the absence of ouabain.

Na^+/K^+ATPase activity was defined as the micrograms of orthophosphate production per milligram of protein at 37°C.

Acid phosphatase, the marker enzyme for lysosomal membranes was assayed using a Sigma Diagnostics Kit (Procedure No. 435) with a computer controlled vertical photometer (Titertek Multiskan MCC/340, Flow Laboratories, McLean, VA). The specific activity of acid phosphatase was expressed as units (U) defined as the amount of enzyme which hydrolyzes one micromole of alpha naphtyl phosphate per minute.

Lactate dehydrogenase (E.C. 1.1.27), the cytosolic marker enzyme, was measured by a modification of the procedure of Storrie and Madden (1990) with a computer controlled vertical photometer (Titertek Multiskan MCC/340, Flow Laboratories, McLean, VA). The decrease in absorbance as a result of conversion of NADH to NAD^+ was monitored at 340 nm. The specific activity was expressed as decrease absorbance units per minute.

Cytochrome c oxidase, the marker enzyme for mitochondria, was analyzed by a modification of the procedure of Storrie and Madden (1990). The decrease in absorbance as a result of oxidation of reduced cytochrome c was measured using a horizontal spectrophotometer at 550 nm. The specific activity was expressed as decrease in absorbance units per minute.

Cytochrome c reductase, the marker enzyme for microsomal membrane, was detected by a modification of the procedure of Master and Williams (1967). The increase in absorbance as a result of reduction of cytochrome c was measured using a horizontal spectrophotometer at 550 nm. The specific activity was expressed as increase in absorbance units per minute.

The BCA method (Pierce BCA Protein Assay Kit 23225, Rackford, IL) was adapted to microtiter plates (Thomas Scientific 913651084) for the determination of protein concentration. Bovine serum albumin was used to establish the standard curve with a range of 0 to 400 ug/mL of protein. All membrane fractions were diluted with .1 N NaOH prior to assay so that the concentration was within the standard curve range.

Transport Assay

On the day of a transport experiment, a suitable number of cryovials containing BBMV were thawed in a water bath (39°C) and suspended in 10 volumes of mannitol transport buffer. Following suspension of BBMV, the vesicles were centrifuged at 105,000 x *g* for 60 min to remove sucrose from the BBMV. Then, the resulting pellet was resuspended with transport buffer to a final protein concentration of 2 to 3 mg/mL and homogenized with a glass-glass homogenizer (Fisher, Tissue grinder, 18 x 130 mm). The BBMV were maintained in a water bath at 39°C throughout the transport experiment.

Methionine and glucose transport by BBMV were characterized as described by Wilson and Webb (1990a). The transport assay buffer contained 20 mM mannitol, 5 mM HEPES, 2 mM $MgCl_2$, and 150 mM of either NaSCN or KSCN (pH; 7.4). The substrate concentrations tested for the measurement of transport kinetics were .1, .2, .4, .8, 1.6, 3.2, and 6.4 mM for methionine and .075, .15, .3, .6, 1.2, 2.4, 4.8, and 9.6 mM for glucose. When transport assay buffers containing these substrate concentrations were prepared, enough mannitol was removed to maintain isoosmolarity; this was verified by an osometer (Osmette A, Precision Systems Inc., MA). The transport reaction was started by adding 125 μL

of brush border membrane suspension to a reaction tube that had been preloaded with 125 μL of transport assay buffer and 125 μL of radioisotope solution containing 2 μCi os substrate preincubated at 39°C. The total reaction volume was 375 μL. For each transport experiment, triplicate reaction tubes were used for measuring each evaluated substrate and six reaction vessels were prepared for determining background binding of filters. The uptake by BBMV was terminated after 15 s of incubation by addition of 1 mL ice cold stopping solution containing .5 mM phloridizin and 150 mM KCl. A 1 mL aliquot from the resulting mixture was immediately filtered through a millipore nitrocellulose filter (.45 um; Millipore Company, MA) and washed three times with 5 mL aliquots of the same ice cold stopping solution to minimize the background binding to the filters. The filter membranes were then placed into 20 mL scintillation vials and 9 mL of scintillation fluid (Fisher Scientific SX 18-4, NJ) were added to each vial. Radioactivity retained within the vesicles on the filters was counted by a scintillation counter (LS5000 TA, Beckman Inc. CA). Radiochemical specific activities for ^{35}S methinone (L-[^{35}S]Methionine, Amersham) and ^{3}H D-glucose (NET-100A Glucose, D-[6-^{3}H(N)]-, Dupont,DE) were 36.2 Ci/mmol and 1,218 Ci/mmol, respectively.

Total uptake of methionine and glucose was partitioned into three components: Na^+-independent uptake, Na^+-dependent uptake, and passive diffusion. Total uptake at each substrate concentration was determined by the incubation of BBMV with transport assay buffer containing NaSCN. The Na^+-independent uptake was calculated as the difference between uptake using the incubation of transport assay buffer containing KSCN and diffusion uptake. The Na^+-dependent uptake was estimated by the difference between uptake measured

with and without Na^+ present. Passive diffusion was measured by determining the permeability constant for methionine and glucose at 100 mM concentration of substrate (Hopfer, 1981).

Statistical Analyses

All data in this experiment were analyzed by the General Linear Model Procedure of SAS (SAS Institute Inc., 1988).

The model for the pig performance trial (experiment 1) was;

$Y_{ijk} = a_i + b_j + r_k(b_j) + a_ib_j + e_{ijk}$

Y_{ijk} = dependent variable

a_i = average effect of diet $_i$

b_j = average effect of group $_j$

$r_k(b_j)$ = pair(group)

a_ib_j = interaction diets*pair

e_{ijk} = error

The model for the lamb performance trial (experiment 2) and mucosal cell weight was;

$Y_{ij} = a_i + b_j + e_{ijk}$

Y_{ij} = dependent variable

a_i = average effect of diet $_i$

b_j = average effect of pair $_j$

e_{ijk} = error

The model for the effect of Aspergillus product on mucosal enzyme activity and enrichment, transport kinetics analyses (K_m and V_{max}) of methionine and glucose (experiment 1 and 2) was;

$$Y_{ijk} = a_i + b_j + r_K + a_ib_j + a_ir_k + b_jr_k + e_{ijk}$$

Y_{ijk} = dependent variable

a_i = average effect of diets $_i$

b_j = average effect of pair $_j$

r_k = average effect of site $_k$

a_ib_j = effect of the interaction diet*pair

a_ir_k = effect of the interaction diet*site

b_jr_k = effect of the interaction pair*site

e_{ijk} = error

The model for the effect of methionine and glucose uptake (experiment 1 and 2) was;

$$Y_{ijk} = a_i + b_j + r_k + a_ir_k + e_{ijk}$$

Y_{ijk} = dependent variable

a_i = average effect of diets $_i$

b_j = average effect of pair $_j$

r_k = average effect of site $_k$

a_ir_k = effect of the interaction diet*site

e_{ijk} = error

Results and Discussion

Growth and Feed Utilization

Experiment 1. Growth and feed utilization data from pigs are presented in Table 3. Feeding an 18% CP corn-soy diet supplemented with .15% *Aspergillus* product to weanling pigs did not improve ($P > .05$) any of the performance criteria measured, including ADG, ADFI, and feed/gain throughout the experimental period. We are unaware of any published report covering the evaluation of *Aspergillus* products in pigs. Probiotics such as bacterial or yeast culture products have been investigated in swine for their possible benefits because they contain substantial amounts of digestive enzymes including cellulases, proteases, amylases, and lipases, and they also contain B vitamins, and minerals (Ustyuzhanina et al., 1984; Kung et al., 1992). If a response to probiotics is observed, this is generally associated with the feeding of probiotics to neonatal or early weaned pigs (Parker, 1990). In the present study, however, there was no response in animal performance to the inclusion of an *Aspergillus* product in the diet of early weaned pigs. When rats and broiler chickens were fed diets containing soybeans fermented with *Aspergillus oryzae*, a significant improvement in ADG and feed conversion was observed compared with animals fed diets containing unfermented soybeans (Chah et al., 1975; Zamora and Veum, 1979). More recently, however, Zamora and Veum (1988) reported that neonatal pigs fed heated whole soybeans fermented with *Aspergillus oryzae* did not have improved ADG, feed efficiency, or CP digestibility compared with pigs fed heated whole soybeans. Supplementation of cellulase, amylase, or protease to grain-based diets

Table 3. Growth and feed utilization of weanling pigs fed an *Aspergillus* product (n=24)

Item	Diet		SE	pa
	Control	Aspergills		
Days 1 to 7				
ADG, g	230	213	8.57	.34
ADFI, g	374	343	11.99	.22
Feed/Gain	1.73	1.66	.05	.54
Days 8 to 14				
ADG, g	321	308	12.96	.63
ADFI, g	588	550	10.40	.11
Feed/Gain	1.87	1.83	.06	.76
Days 15 to 21				
ADG, g	434	409	15.02	.43
ADFI, g	757	703	17.91	.17
Feed/Gain	1.75	1.81	.06	.69
Days 22 to 28				
ADG, g	460	478	13.88	.50
ADFI, g	871	879	18.70	.85
Feed/Gain	1.94	1.86	.06	.38
Days 29 to 35				
ADG, g	549	560	28.79	.86
ADFI, g	1083	1080	33.53	.97
Feed/Gain	2.0	1.96	.06	.75
Days 1 to 35				
Initial BW,Kg	6.75	6.70	.05	.57
Final BW,Kg	20.16	19.91	.30	.69
ADG, g	394	389	8.77	.75
ADFI, g	724	700	15.41	.45
Feed/Gain	1.85	1.80	.01	.15

[a]Probability that a difference between diets this large or larger could be have occurred by chance.

has been shown to improve feed utilization and growth rate of pigs and ckickens (Suga et al., 1978). In other studies, the addition of these enzyme preparations failed to improve animal performance (Moss et al., 1977; Reese et al., 1983).

Experiment 2. Feeding the *Aspergillus* product to lambs did not alter overall ADG, feed intake, or feed utilization for the 91-d feeding period (Table 4). Initial performance of lambs fed the *Aspergillus* product was not as good as the control lambs as evidenced by the fact that, through 21-d of the feeding period, cumulative ADG (data not shown) of the lambs fed the *Aspergillus* product was lower ($P < .05$) than the control lambs. Herring and Hallford (1991) reported that neither feed intake, gain, nor feed/gain of lambs was altered by a diet containing pelleted alfalfa and rolled corn supplemented with .3 g/d or .6 g/d *Aspergillus* product. Denigan et al. (1992) reported that the inclusion of *Aspergillus* to a 60% concentrate diet in dairy cows did not improved gain, feed intake, or feed efficiency. Likewise in other studies, no differences in milk yield or fat corrected milk in dairy cows was observed (Gomez-Alarcon et al., 1990; Kellems et al., 1990; Higginbothan et al., 1993). Furthermore, several studies failed to demonstrate any alteration in fiber digestion (NDF, ADF, cellulose), CP digestion or DM digestion in response to *Aspergillus* products (Firkins et al., 1990; Denigan et al., 1992; Oellermann et al., 1990). In contrast to these results, other researchers have reported that moderate and high concentrate diets fortified with *Aspergillus* products improved ADG and feed efficiency, in cattle (Hatfield and Hixon, 1975), steers (Slippers et al., 1988), and milk yield in dairy cows (Gomez-Alarcon et al., 1991). Also, Wiedmeier et al. (1987) observed an improved digestion of DM, hemicellulose, and CP in dairy cows when a 50% concentrate diet was

Table 4. Growth and feed utilization of growing lambs fed an *Aspergillus* product (n=24)

Item	Diet		SE	pa
	Control	Aspergillls		
Days 1 to 7				
ADG, g	477	451	16.9	.46
ADFI, g	1,034	1,020	13.2	.60
Feed/Gain	2.3	2.34	.08	.61
Days 8 to 14				
ADG, g	310	278	19.7	.44
ADFI, g	1,283	1,234	23.1	.31
Feed/Gain	4.7	5.0	.37	.70
Days 15 to 21				
ADG, g	343	335	14.8	.80
ADFI, g	1,351	1,306	28.4	.45
Feed/Gain	4.2	4.4	.34	.48
Days 22 to 28				
ADG, g	236	246	12.8	.70
ADFI, g	1,418	1,336	35.5	.28
Feed/Gain	6.8	5.8	.37	.19
Days 29 to 35				
ADG, g	244	241	15.0	.94
ADFI, g	1,445	1,397	26.4	.39
Feed/Gain	6.5	7.0	.73	.74
Days 36 to 42				
ADG, g	219	222	14.9	.94
ADFI, g	1,424	1,385	28.5	.52
Feed/Gain	6.7	7.0	.53	1.0
Days 43 to 49				
ADG, g	229	215	18.1	.72
ADFI, g	1,466	1,367	31.3	.15
Feed/Gain	8.0	5.0	1.29	.27
Days 50 to 56				
ADG, g	86	122	17.1	.32
ADFI, g	1,416	1,306	34.0	.96
Feed/Gain	12.1	13.3	1.51	.71
Days 57 to 63				
ADG, g	112	177	29.1	.29
ADFI, g	1,420	1,547	65.6	.36
Feed/Gain	6.5	9.8	.95	.11

Days 64 to 70				
ADG, g	202	227	14.4	.43
ADFI,g	1,305	1,383	45.2	.43
Feed/Gain	7.9	7.1	.44	.42
Days 71 to 77				
ADG, g	192	224	15.5	.35
ADFI, g	1,339	1,374	39.0	.68
Feed/Gain	7.6	6.4	.38	.20
Days 78 to 84				
ADG, g	165	135	15.8	.40
ADFI, g	1,324	1,321	29.2	.97
Feed/Gain	10.2	7.9	1.66	.14
Days 85 to 91				
ADG, g	158	172	9.7	.51
ADFI, g	1,347	1,264	29.6	.21
Feed/Gain	11.2	8.9	.67	.14
Days 1 to 91				
Initial BW,Kg	21.8	22.1	.15	.36
Final BW,Kg	43.1	43.5	.60	.74
ADG, g	227	234	7.0	.63
ADFI, g	1,342	1,345	24.4	96
Feed/Gain	6.0	5.8	.13	.62

[a]Probability that a difference between diets this large or larger could be have occurred by chance.

supplemented with 2.63g/d *Aspergillus* product. In total, the results of research to date are inconclusive on the effect of *Aspergillus* product on animal production. Responses seem to vary according to dietary fiber source (or fiber type), relative percentage of concentrate in the diet, level of *Aspergillus* product supplementation, stage of production, and (or) animal age.

Characterization of Brush Border Membrane Vesicles

To identify the purity of the intestinal BBMV derived from the pigs and lambs, the enrichment of several enterocyte organelle marker enzymes was determined. The procedure for isolation of BBMV was a slight modification of that from Wilson and Webb (1990b). The method of isolation of BBMV included Mg^{++} diavalent cation incubation, differential centrifugation, and density gradient ultracentrifugation as mentioned earlier.

Experiment 1. The percentage recoveries at all the differentiation steps of protein and marker enzyme activities are presented in Appendix Tables 1 to 8. Of the original protein content of the mucosal homogenate, less than .4% was recovered as isolated BBMV in band 1 and band 2 (Appendix Table 1). There was no apparent problem with inactivation of marker enzymes throughout the fractionation process as indicated by the recovery from one step to another (Appendix Tables 2 to 8). The isolation process resulted in a membrane preparation that was rich in brush border membrane with minimal contamination with other cellular organelles (Table 5). The enrichments of both alkaline phosphatase and sucrase were quite high indicating a marked concentration of brush border membrane in comparison with the original homogenate. The minimal

Table 5. The degree of purification of isolated brush border membrane vesicles in terms of enzyme enrichment in pigs (n=4)[a]

Enzymes	Brush border membrane	
	Band 1	Band 2
Alkaline phosphatase	15.40	17.57
Sucrase	10.74	15.41
Na^+/K^+ATPase	2.39	2.78
Acid phosphatase	2.87	1.97
Cytochrome c oxidase	.08	.06
Cytochrome c reductase	2.79	2.73
Lactate dehydrogenase	.66	.48

[a]Data are calculated as the specific activity of the enzyme in the BBMV divided by the specific activity of the enzyme in the original homogenate.

enrichment of other marker enzymes indicates minimal contamination with other cellular organelles. Of particular interest is Na^+/K^+ATPase, the marker enzyme for basolateral membrane. Because the basolateral membrane must be sheered free from the brush border membrane, it is important that the homogenization process produces small fragments of membrane. The small enrichment of Na^+/K^+ATPase observed relative to the enrichment of alkaline phosphatase indicates that the our procedures resulted in only a small amount of basolateral membrane being isolated with the brush border membrane.

Overall, the isolation procedure yielded highly purified BBMV that exhibited negligible contamination with other cellular organelles. In other studies with various species including pigs, rats, guinea pigs, cat, and humans, a 7- to 20-fold enrichment of alkaline phosphatase, and a 1- to 2-fold enrichment of Na^+/K^+ATPase have been reported (Wolffram, et al., 1986, 1989; Malo and Berteloot, 1991; Satoh et al., 1991).

Experiment 2. The percentage recoveries at all the differentiation steps of protein and marker enzyme activities are presented in Appendix Tables 9 to 15. Only a very small portion of the original starting protein (< .4%) was recovered in the isolated BBMV (Appendix Table 9). Recovery of marker enzymes from one step to another during the fractionation process indicated that no problem existed with enzyme inactivation. Enrichment of alkaline phosphatase averaged about 6.6-fold (mean of band 1 and 2; Table 6). This is less than the average of 16.5-fold observed in isolated BBMV from pig small intestine(Table 5). However, it is similar to the 5.1-fold enrichment in alkaline phosphatase activity observed in BBMV isolated from the small intestine of steers (Wilson and Webb, 1990b) and

Table 6. The degree of purification of isolated brush border membrane vesicles in terms of enzyme enrichment in lambs (n=4)[a]

Enzymes	Brush border membrane	
	Band 1	Band 2
Alkaline phosphatase	5.13	8.11
Na^+/K^+ATPase	1.47	1.64
Acid phosphatase	2.24	2.20
Cytochrome c oxidase	.13	.12
Cytochrome c reductase	1.14	1.46
Lactate dehydrogenase	.68	.56

[a]Data are calculated as the specific activity of the enzyme in the BBMV divided by the specific activity of the enzyme in the original homogenate.

the 7-fold enrichment observed in cows (Moe et al., 1985). Contamination of the BBMV with other cellular organelles was minimal (Table 6). With the exception of acid phosphatase, enrichments of all marker enzymes were less than 2-fold. The average enrichment of Na^+/K^+ATPase of 1.6-fold observed in the present study was similar to the 1.3-fold enrichment observed in BBMV from steers (Wilson and Webb, 1990b). The similarity of marker enzyme enrichments observed in the present study with sheep and the previous work with steers and the different enrichment observed with pigs and other monogastric species may indicate that the physical properties and enzyme composition of ruminant small intestinal brush border membrane varies from other species. However, Wolffram et al. (1986) observed a 15- to 17-fold enrichment in alkaline phosphatase in BBMV prepared from both sheep and pigs.

In Experiments 1 and 2, the characterization of the process of isolation of BBMV from the intestine of pigs and lambs clearly demonstrated that the isolated BBMV can be considered as adequate for the purpose of studying methionine and glucose transport.

Although, the enrichment of marker enzymes in the intestinal BBMV was essential to confirm the purity of the membrane preparations, these criteria do not always guarantee that the membrane vesicles are appropriate for assessing transport properties of the isolated membrane. Isolated BBMV preparations must also be examined in terms of both vesicle size and their capacities for transport. These criteria were evaluated in our laboratory previously by Wilson and Webb (1990b). They reported that the bovine BBMV prepared from a very similar isolation procedure with the present procedure gave rise to membranes of a vesicular nature as observed under electron microscopy. Also, they varified the

vesicular nature of the isolated BBMV by characterizing substrate uptake under varying osmotic conditions.

Working with membrane vesicles has been identified as having great merit for overcoming the problems of other in vitro and in vivo methods such as stirred layers, paracellular absorption routes, and metabolism of transported solutes by the intestinal cells (Argiles and Lopez-Soriano, 1990). There are also short comings one must consider when interpreting the results from the use of isolated BBMV. The BBMV may exhibit a "leaky" nature because of increased permeability toward transport solutes compared with the original membrane due to vesicles formed from disrupted membrane. Consequently, detection of a low uptake when measuring diffusion might be difficult. Another potential disadvantage is that very short incubation times are required for the determination of unidirectional fluxes because of the small intravesicular space (Kessler and Toggenburger, 1979).

Mucosal Weight and Sucrase, Alkaline Phosphatase, and Na^+/K^+ATPase Activities

Experiment 1. No significant diet-dependent difference in jejunal, ileal, or total wet mucosal weight of pigs were detected (Table 7). However, the ileal wet mucosal weight of pigs fed the *Aspergillus* product tended ($P < .12$) to be less than that of pigs fed the control diet. Specific activities of sucrase, alkaline phosphatase, and Na^+/K^+ATPase of intestinal mucosal cells in pigs were not influenced ($P > .05$) by the feeding of an *Aspergillus* product (Table 8). Furthermore, intestinal site did not influence ($P > .05$) the specific activities of

Table 7. Weights of mucosal tissue isolated from pigs fed an *Aspergillus* product[a] (n=12)

Mucosa	Diet		SE	p[b]
	Control	Aspergillus		
	g			
Jejunum	292.1	270.7	14.7	.50
Ileum	252.3	221.9	8.0	.12
Total	544.3	492.6	21.4	.29

[a]Wet basis.
[b]Probability that a difference between diets this large or larger could have occurred by chance.

Table 8. Specific activities of sucrase, alkaline phosphatase, and Na^+/K^+ ATPase in intestinal mucosal tissue from pigs fed an *Aspergillus* product

	Diet							
	Control		Aspergillus					
Enzyme	Jejunum	Ileum	Jejunum	Ileum	SE	p^a	p^b	p^c
Sucrase (μmol/mg/min)	7.57	8.11	7.01	8.51	.29	.90	.15	.46
Alkaline phosphatase (μmol/mg/min)	.071	.058	.060	.065	.002	.67	.49	.14
Na^+/K^+ATPase (U)[d]	.040	.044	.041	.041	.002	.19	.21	.39

[a]Probability that a difference between diets this large or larger could have occurred by chance.
[b]Probability that a difference between sites this large or larger could have occurred by chance.
[c]Probability that the diet*site interaction that was observed could have occurred by chance.
[d]One U is defined as the amount of Na^+/K^+ATPase which will liberate 1.0 μmole of inorganic phosphate from ATP per min at pH 7.4 and 37^0C.

these enzymes. However, sucrase activity tended ($P < .15$) to be higher in the ileum than the jejunum.

Experiment 2. Weights of mucosal tissue collected from lambs are presented in Table 9. The jejunal mucosal weight of jejunum from lambs fed the *Aspergillus* product was about 13 % less ($P < .08$) than from lambs fed the control diet. There was a tendency ($P < .12$) for total mucosal tissue weight to be less when the *Aspergillus* product was fed to lambs. Parker and Armstrong (1987) reported in a review that altering the microbial population of the small intestine such as in germ-free or gnotobiotic animals or in animals fed antibiotics often results in changes in the histology of the villi of the small intestine. Neither morphological nor histological examinations were performed on the small intestine, so potential for structure changes to have occurred can not be addressed. The specific activities of alkaline phosphatase and Na^+/K^+ATPase were not affected by the feeding of the *Aspergillus* product (Table 10). Alkaline phosphatase specific activity was about five times greater ($P < .0004$) for jejunal mucosa compared with ileal mucosa. The specific activity of Na^+/K^+ATPase remained essentially constant along the entire length of the small intestine.

Mucosal cell weight may be associated with expression of intestinal absorptive function, but it also may be affected by other factors such as genetic variability and age. McCarthy et al. (1980) reported that feeding a high protein diet to rats resulted in an increased duodenal mucosal weight. However, mice fed different dietary amino acids and proteins did not exhibit diet-dependent differences in intestinal length or wet mucosal weight, although transport

Table 9 Weights of mucosal tissue weights isolated from lambs fed an*Aspergillus* product[a] (n=12)

Mucosa	Diet		SE	p[b]
	Control	Aspergillus		
	g			
Jejunum	211.8	184.4	6.2	.08
Ileum	204.1	209.1	7.2	.75
Total	415.9	393.5	5.9	.12

[a]Wet basis.
[b]Probability that a difference between diets this large or larger could have occurred by chance.

Table 10. Specific activities of alkaline phosphatase and Na^+/K^+ATPase in intestinal mucosal tissue from lambs fed an *Aspergillus* product

	Diet							
	Control		Aspergillus					
Enzyme	Jejunum	Ileum	Jejunum	Ileum	SE	p[a]	p[b]	p[c]
Alkaline phosphatase (μmol/mg/min)	.279	.061	.271	.047	.03	.69	.0004	.92
Na^+/K^+ATPase (U)[d]	.025	.028	.028	.027	.027	.31	.41	.22

[a]Probability that a difference between diets this large or larger could have occurred by chance.
[b]Probability that a difference between diets this large or larger could have occurred by chance.
[c]Probability that the diet*site interaction that was observed could have occurred by chance.
[d]One U is defined as the amount of Na^+/K^+-ATPase which will liberate 1.0 umole of inorganic phosphate from ATP per min at pH 7.4 and 37°C.

capability of amino acids by the intestine was increased by dietary level of amino acids and protein (Stein et al., 1987).

It is likely that enterocyte migration rate or turnover rate and crypt-villus morphology are important factors that influence the effect of dietary modulation on intestinal morphology. Apparently, slow cell migration rate in pigs enhances the intestinal hydrolase activity and functional capacity of the villus surface for alanine transport due to a more mature villus population (Miller et al., 1986). Parker (1990) reported that the supplementation of avoparcin, an antibiotic feed additive, markedly reduced the mucosal cell division rate in the crypts of duodenal tissue, suggesting that the inclusion of antibiotics may impact on the expression of intestinal transport proteins.

To identify the effect of the *Aspergillus* product on enterocyte maturation (cell migration rate) indirectly, specific activities of alkaline phosphatase and sucrase, which are exclusively limited to the microvillus membrane (brush border membrane) in the intestine, were quantified. These enzymes have been shown to exhibit a gradient along the crypt-villus axis or the intestinal length in the mice (Miller et al., 1986; Ferraris and Diamond, 1992). Sucrase is a marker enzyme for cell differentiation (Kretchmer et al, 1979). Alkaline phosphatase, also an important constituent of the microvillus membrane, has not been clearly defined with regards to its function. Alkaline phosphatase may be involved in dephosphorylation of phosphoprotein and other dietary components under physiological conditions (Tuba and Dickie, 1955; McCarthy et al., 1980). Like sucrase and other microvillus enzymes, it is assumed that alkaline phosphatase activity may be related to the synthesis of cellular proteins, differentiation of the cell, and proper glycosylation in the enterocytes (Moong, 1979). Ferraris et al.

(1992a) reported that sucrase, alkaline phosphatase, and gamma-glutamyl transpeptidase (GGP) located in the intestinal brush border membrane, enhanced their activities in response to increasing dietary carbohydrate, protein, or sucrose levels.

Intestinal sucrase, alkaline phosphatase, and maltase activities also were increased by feeding a high fat and cholesterol diet to monkeys (Dhaunsi et al., 1989). The inclusion of probiotics (lactobacillus) or antibiotics (tylosin) to weanling pigs (17d age) had a marked effect on the development of sucrase, lactase, and tripeptidase activities (Collington et al., 1990). However, like in the present study, neither antibiotics nor probiotics had a significant impact on the expression of sucrase, alkaline phosphatase, lactase, or peptidase activities at 42 and 80 d of age (Collington et al., 1990). Shirazi-Beechey et al. (1991b) pointed out that the development of alkaline phosphatase, lactase, and maltase was not regulated by dietary factors in lambs. An intrinsic mechanism rather than alteration of diet composition may be more responsible for altered expression of sucrase in rats and piglets (Yeh and Holt, 1986; James et al., 1987).

As regards intestinal site differences for alkaline phosphatase and sucrase activity, regional differences along the small intestine are known to occur (McCarthy et al., 1980; Hampson and Smith, 1986). It also seems that regional differences in these enzymes may depend on species. Generally, in the small intestine of mice, alkaline phosphatase activity declines steeply from the duodenum to the ileum, and sucrase activity peaks in the lower duodenum and upper jejunum (Moog, 1977). Fearris et al. (1992a) reported that the duodenum of mice possessed an almost 10-fold greater specific activity of alkaline phosphatase than the ileum. They also observed that the highest sucrase activity occurred in the

duodenum and upper jejunum. In our results with lamb intestine, a 5-fold greater alkaline phosphatase activity was observed in the jejunum compared with the ileum.

In monkeys, V_{max} of alkaline phosphatase and sucrase was 1.5- and 1.74-fold greater, respectively, in the jejunum compared with the ileum (Dhaunsi et al., 1989). In contrast to site differences of sucrase activity reported for mice, examination of the intestine of growing pigs showed that sucrase activity of the distal intestine was higher than that of the proximal intestine. (Collington et al., 1990). Our observations with pigs in the present study are similar. Collington et al. (1990) measured sucrase activity at five positions according to intestinal length and observed a gradual increase in sucrase activity from the proximal to the distal site. The dwindling sucrase activity of "proximal to distal" along rat intestine or "distal to proximal" along pig intestine is presumably associated with the presence of luminal glucose (or sucrose) as well as the ability of sucrose hydrolysis into glucose and glucose absorption along the intestine (Ferraris et al., 1990, 1992a).

Na^+/K^+ATPase, an intrinsic plasma membrane protein, plays an important role in transporting most amino acids and glucose under physiological circumstances (Garnner, 1990). Therefore, differences in the activity of Na^+/K^+ATPase could reflect an altered Na^+-dependent, energy requiring transport mechanism (Darnell et al., 1990). It is well known that ionophores such as monensin and lasolocid stimulate Na^+/K^+ATPase activity and subsequently elevate Na^+-dependent, active transport at the cellular level of the small intestine. However, there was no direct evidence from the present study or others that probiotics, including fungal or yeast extracts, can affect the activity of Na^+/K^+ATPase.

Marker Enzyme Enrichment in BBMV Preparations used in the Transport Study

Previously described marker enzyme enrichment was a discussion about the data collected during the characterization of the recovery of marker enzymes. Subsequent to establishing the validity of the methodology, BBMV for use in transport studies were prepared from jejunal and ileal tissue collected from lambs and pigs fed either a control diet or the same diet supplemented with an *Aspergillus* product. Within each species, BBMV were prepared from six animals receiving each treatment. The data for enzyme enrichment in the intestinal BBMV from pigs and lambs used for methionine and glucose transport evaluations are presented in Tables 11 and 12.

Experiment 1. The enrichment of sucrase and alkaline phosphatase were similar and indicative of a high degree of brush border membrane concentration in the preparation from pigs (Table 11). Sucrase enrichments were similar between diets, however, alkaline phosphatase enrichment in BBMV from pigs fed the *Aspergillus* product was about 25 % greater ($P < .14$) than in pigs fed the control diet. Enrichment of sucrase was greater ($P < .02$) in ileal BBMV than jejunal BBMV. There was only a modest enrichment, usually less than 2-fold, in Na^+/K^+ATPase activity. This indicates a minimal contamination with basolateral membrane.

Experiment 2. Sheep do not have the sucrase enzyme, therefore, alkaline phosphatase was the sole marker enzyme for the brush border membrane. The enrichment of alkaline phosphatase was about 12 % greater ($P < .08$) in BBMV from lambs fed the control diet and about 15 % greater ($P < .05$) in BBMV from

Table 11. Enrichment of sucrase, alkaline phosphtase, and Na^+/K^+ATPase in brush border membrane vesicles prepared from the intestine of pigs fed an *Aspergillus* product (n=6)[a]

	Diet							
	Control		Aspergillus					
Enzyme	Jejunum	Ileum	Jejunum	Ileum	SE	p[b]	p[c]	p[d]
Sucrase	10.92	14.35	12.68	13.28	.39	.20	.02	.77
Alkaline phosphatase	11.26	11.29	13.08	15.28	.59	.14	.83	.83
Na^+/K^+ATPase	2.02	1.60	1.62	1.72	.07	.40	.31	.14

[a]Data expressed as multiples of activity in the original homogenate.
[b]Probability that a difference between diets this large or larger could have occurred by chance.
[c]Probability that a difference between sites this large or larger could have occurred by chance.
[d]Probability that the diet*site interaction that was observed could have occurred by chance.

Table 12. Enrichment of alkaline phosphatase and Na^+/K^+ATPase in brush border membrane vesicles prepared from the intestine of lambs fed an *Aspergillus* product (n=6)[a]

	Diet							
	Control		Aspergillus					
Enzyme	Jejunum	Ileum	Jejunum	Ileum	SE	p^b	p^c	p^d
Alkaline phosphatase	6.52	5.37	5.49	5.06	.15	.08	.05	.29
Na^+/K^+ATPase	1.23	1.16	1.06	.97	.10	.40	.71	.96

[a]Data expressed as multiples of activity in the original homogenate.
[b]Probability that a difference between diets this large or larger could have occurred by chance.
[c]Probability that a difference between sites this large or larger could have occurred by chance.
[d]Probability that the diet*site interaction that was observed could have occurred by chance.

the jejunum. Enrichment of Na^+/K^+ATPase was essentially non existent indicating minimal contamination with basolateral membrane.

The reason for the possible effects of diet on the enrichment of alkaline phosphatase in BBMV is not obvious. Furthermore, it is not apparent why there was more ($P < .14$) enrichment of alkaline phosphatase when *Aspergillus* was fed to pigs and less ($P < .08$) enrichment of alkaline phosphatase when Aspergillus was fed to lambs. The differences noted here due to diet and intestinal location must be considered as transport results are interpreted.

Methionine and Glucose Transport

Because the small intestine is capable of adapting its nutrient transport to various luminal environmental circumstances and because intestinal nutrient transporters may be regulated by combinations of dietary factors, methionine and glucose uptake by isolated intestinal BBMV from pigs and lambs fed an *Aspergillus* product were studied. Total uptake, carrier-mediated uptake and simple diffusion were quantified at seven different substrate concentrations for methionine and eight different substrate concentrations for glucose, respectively. Carrier mediated uptake was partitioned into Na^+-dependent and Na^+-independent uptake and transport kinetics were determined for these parameters.

Experiment 1. Overall, total uptake, Na^+-dependent uptake, Na^+-independent uptake, and diffusion of methionine increased with increasing substrate concentration and none were influenced ($P > .05$) by the inclusion of the *Aspergillus* product in the diet (Figures 1 and 2; Appendix Table 16). The Na^+-independent uptake of methionine by the intestinal BBMV from pigs fed the

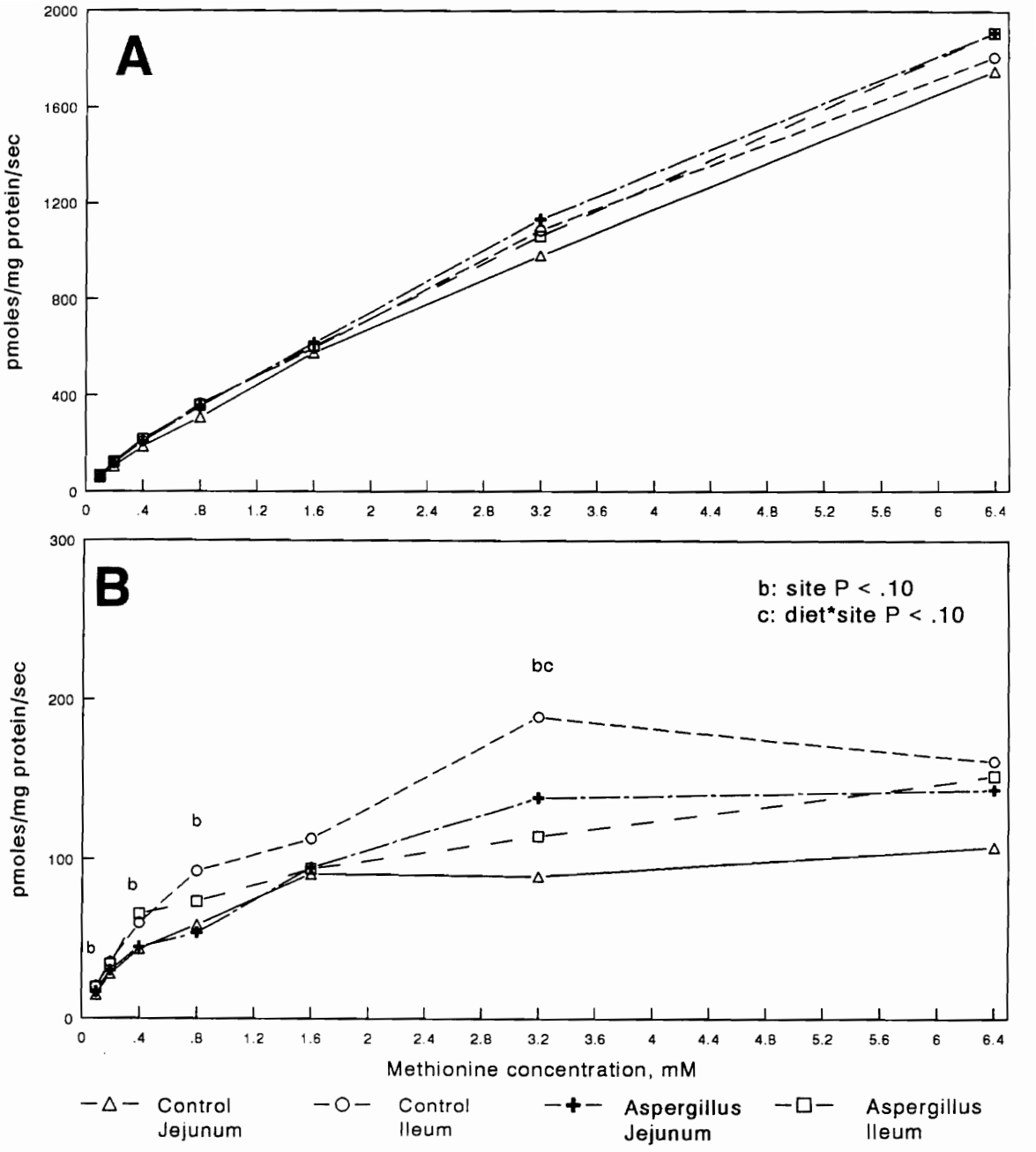

Figure 1. Total uptake (A) and Na^+-dependent uptake (B) of methionine by jejunal and ileal BBMV from pigs fed a control diet and the control diet supplemented with an *Aspergillus* product. All data values are means from six animals. The SE for concentrations of .1, .2, .4, .8, 1.6, 3.2, and 6.4 mM were 1.8, 3.7, 6.9, 8.0, 15.3, 32.5, 43.7 and 1.2, 2.7, 5.0, 6.5, 10.0, 17.6 pmoles/mg protein/sec for total uptake and Na^+-dependent uptake, respectively.

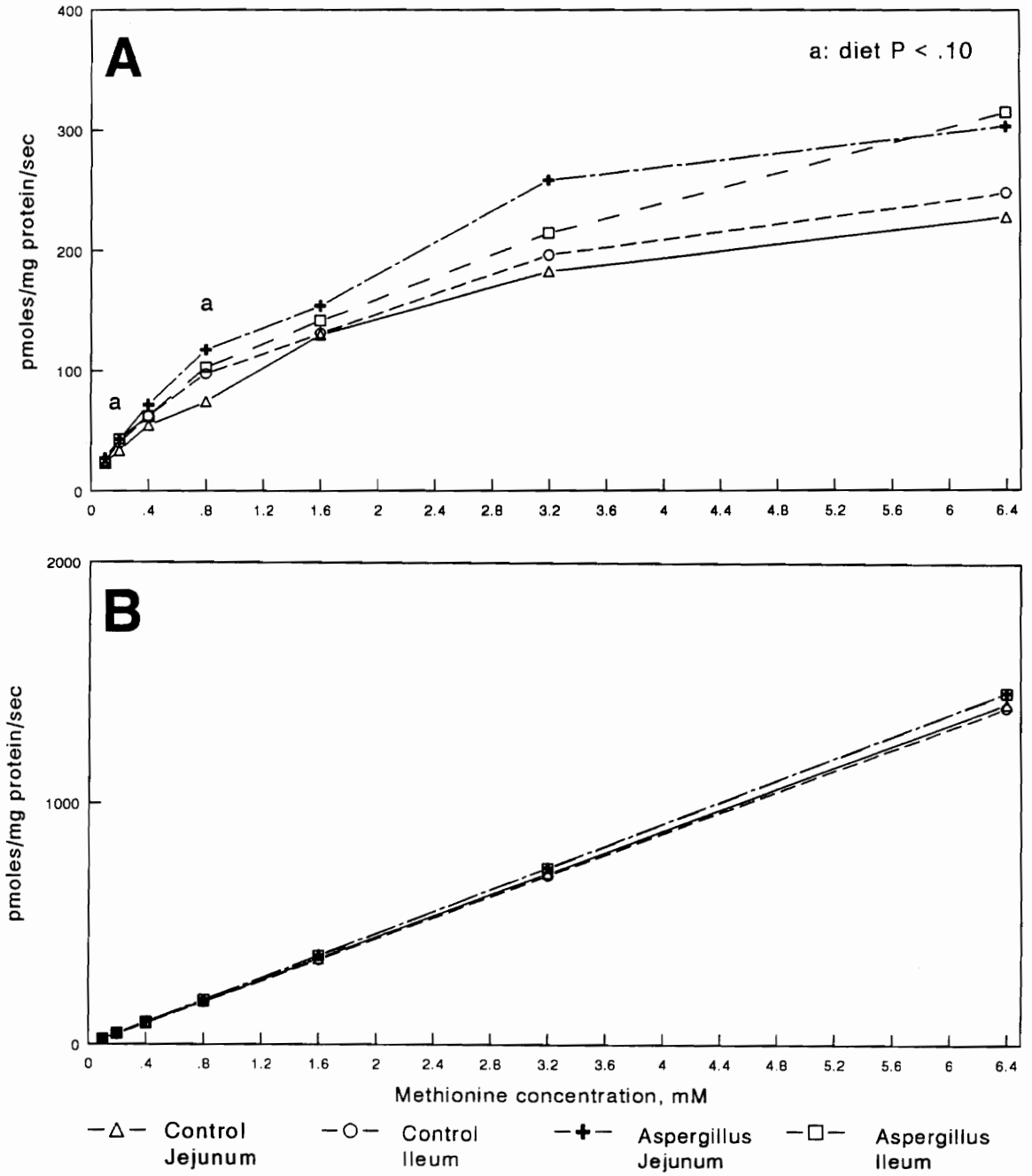

Figure 2. Na^+-independent uptake (A) and diffusion (B) of methionine by jejunal and ileal BBMV from pigs fed a control diet and the control diet supplemented with an *Aspergillus* product. All data values are means from six animals. The SE for concentrations of .1, .2, .4, .8, 1.6, 3.2, and 6.4 mM were .8, 1.4, 2.8, 6.8, 11.0, 20.0, 26.6 and .6, 1.2, 2.4, 4.8, 9.7, 19.4, 38.7 pmoles/mg protein/sec for Na^+-independent uptake and diffusion, respectively.

Aspergillus product was greater ($P < .10$) at .2 and .8 mM methionine concentration and numerically greater at all methionine concentrations evaluated compared with pigs fed the control diet. The apparent K_m and V_{max} values for Na^+-dependent and Na+-independent uptake of methionine by pigs fed the control diet were similar ($P > .05$) to the values for pigs fed the *Aspergillus* product (Table 13).

As with methionine, total uptake, Na^+-dependent, and Na^+-independent uptake, and diffusion of glucose increased with increasing substrate concentration and was not significantly ($P > .05$) altered in response to the inclusion of the *Aspergillus* product in the diet (Figures 3 and 4; Appendix Table 18). The apparent K_m and V_{max} values for Na^+-dependent and Na^+-independent glucose uptake by the jejunal and ileal BBMV from pigs fed the control diet were similar ($P > .05$) to the values from pigs fed the *Aspergillus* product (Table 13). When comparing Na^+-dependent with Na^+-independent glucose transport, the Na^+-dependent transport showed a higher affinity and a lower capacity than the Na^+-independent system.

No information is available regarding the influence of *Aspergillus* products on nutrient transport in pig intestine. The ability of intestinal mucosal cells to adapt to changes in dietary substrate has been recognized for many years. *Aspergillus* products and other nonbacterial probiotics may contain substantial amounts of cellulase, amylase, protease, and lipase activity along with B vitamins (Boing, 1983; Kung et al., 1992). Earlier studies reported that the inclusion of cellulase, amylase, or protease improved nutrient absorption via the increase in hydrolysis of plant structure or the decrease in the viscosity in the intestine in pigs and chicks fed diet containing high soluble glucan chain components (Burnett,

1966: White et al., 1981). While in contrast, several other studies failed to demonstrate the improvement of nutrient digestibility and utilization (Bodart, 1975: Goh et al., 1982: Reese et al., 1983). No studies have been reported where intestinal capacity to deal with nutrient transport in response to the inclusion of enzyme products was evaluated.

In other aspects, the feeding of antibiotics and probiotics altered the digestive enzyme profile along the small intestine, suggesting that these feed additives may be associated with the expression of mucosal cell function (Parker, 1990). An antibiotic, Virginiamycin, has been shown to enhance uptake of free essential amino acids by isolated intestinal loops in pigs (Dierick et al., 1986). Sodium-dependent uptake of methionine was generally greatest in ileal BBMV (Figure 1; Appendix Table 16). Sodium-independent uptake and diffusion were similar between jejunal and ileal BBMV (Figure 2; Appendix Table 16).

For all modes of glucose uptake, there appeared to be little influence of intestinal site. Uptake of glucose by both ileal and jejunal BBMV was generally similar within a transport mechanism (Figures 3 and 4; Appendix Table 18).

The middle region of the small intestine for amino acids and the upper to middle jejunum for glucose in species such as rats, guinea pigs, and humans is usually recognized as having the greatest potential transport capability (Spencer and Samiy, 1961; Cohen and Huang, 1964;, Matthews and Laster, 1965; Gray, 1981; Karasov et al., 1983; Harig et al., 1989). In the present experiment, the small intestine was divided into halves. Therefore, our results showing the similar capacity of methionine transport in the jejunum and ileum is likely due to the inability to differentiate between the middle and the ends of the small intestine. However, glucose uptake in our data with pigs seems to be different from other

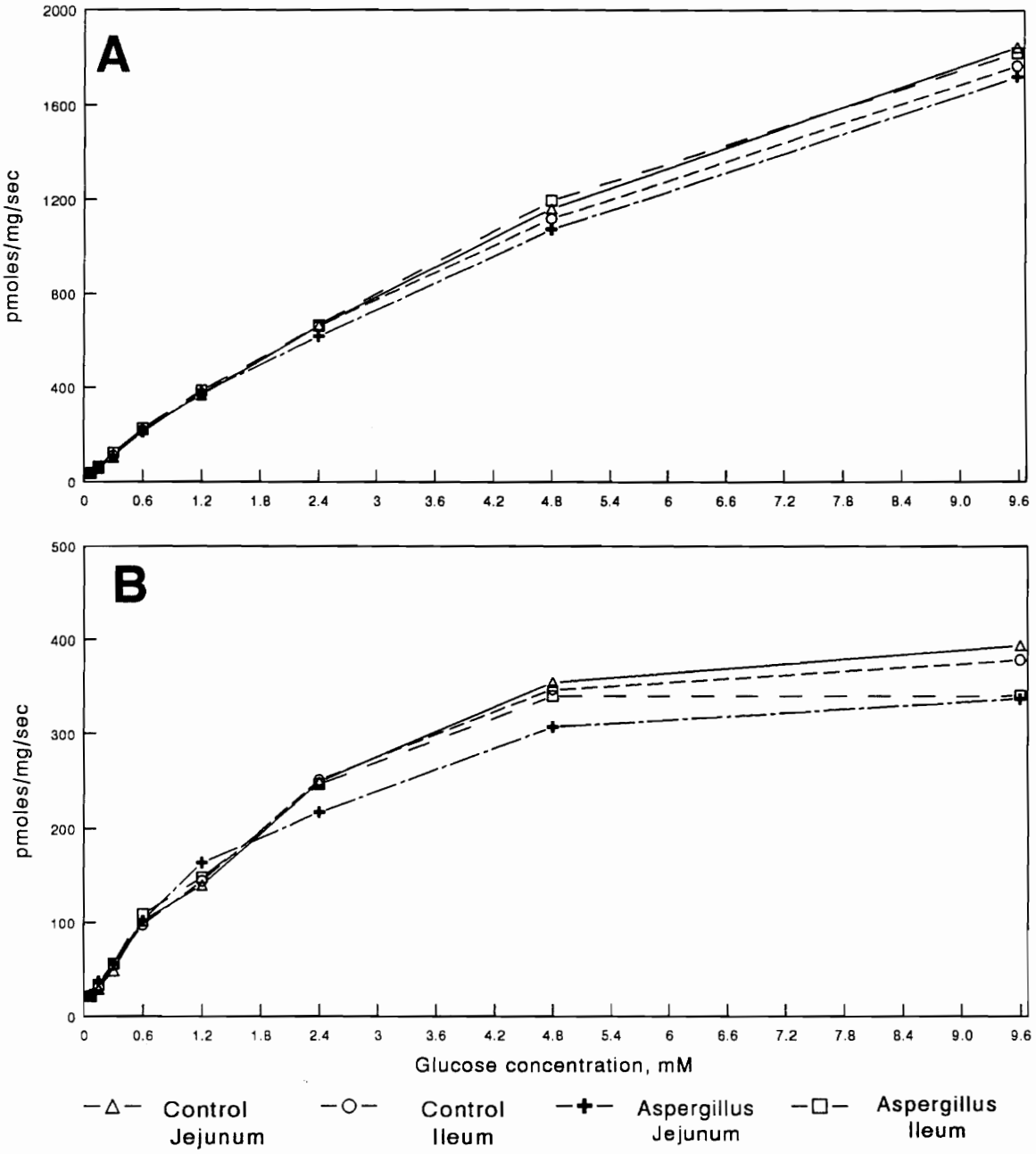

Figure 3. Total uptake (A) and Na^+-dependent uptake (B) of glucose by jejunal and ileal BBMV from pigs fed a control diet and the control diet supplemented with an *Aspergillus* product. All data values are means from six animals. The SE for concentrations of .075, .15, .3, .6, 1.2, 2.4, 4.8, and 9.6 mM were 1.8, 3.0, 4.9, 9.8, 15.4, 23.6, 38.8, 47.4 and 1.6, 2.5, 4.4, 7.2, 11.4, 18.3, 24.8, 29.3 pmoles/mg protein/sec for total uptake and Na^+-dependent uptake, respectively.

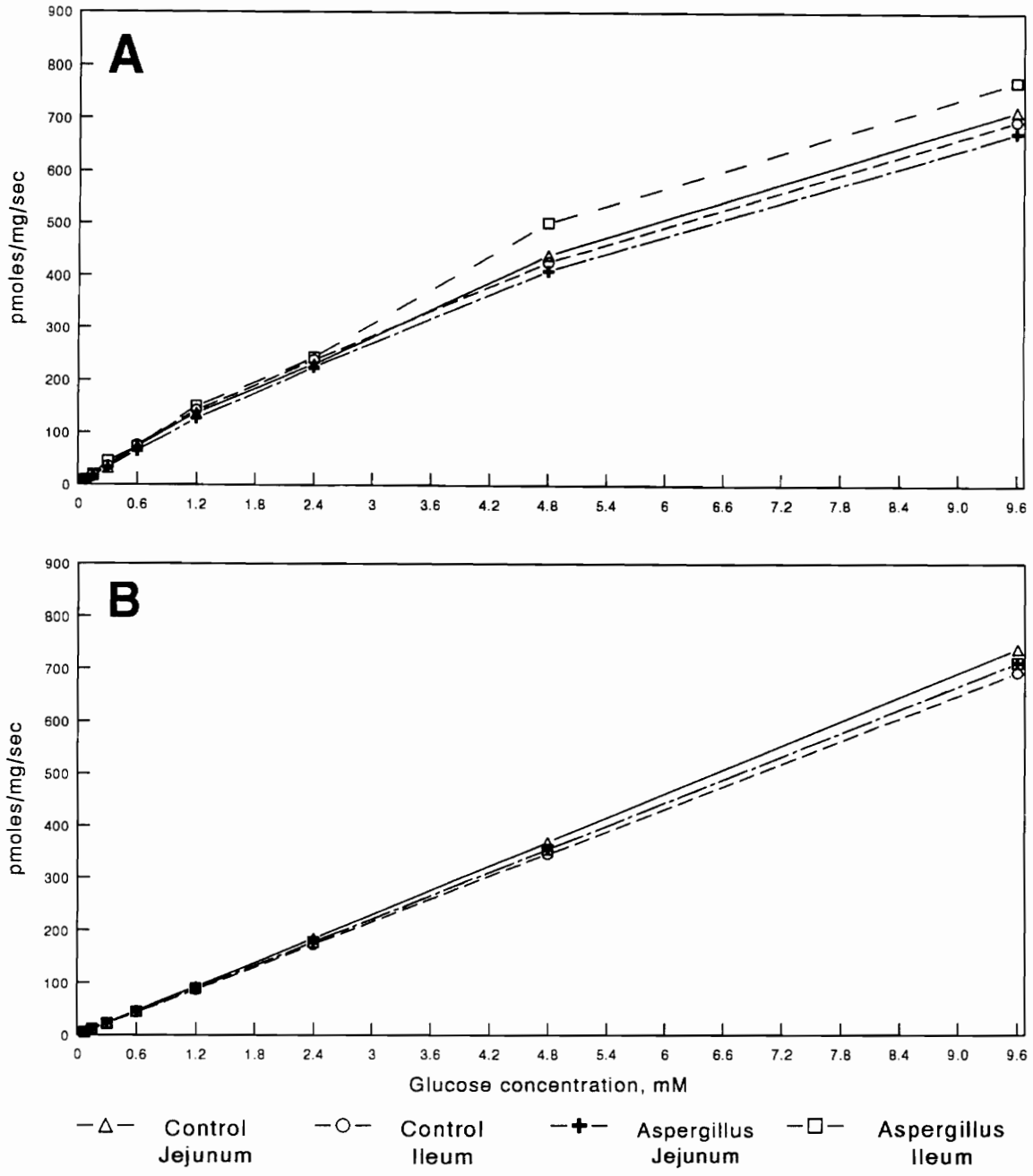

Figure 4. Na^+-independent uptake (A) and diffusion (B) of glucose by jejunal and ileal BBMV from pigs fed a control diet and the control diet supplemented with an *Aspergillus* product. All data values are means from six animals. The SE for concentrations of .075, .15, .3, .6, 1.2, 2.4, 4.8, and 9.6 mM were .4, .6, 2.5, 2.5, 6.1, 8.7, 14.0. 27.8 and .2, .3, .6, 1.2, 2.5, 4.9, 9.9, 19.8 pmoles/mg protein/sec for Na^+-independent uptake and diffusion, respectively.

Table 13. The K_m and V_{max} of Na^+-dependent and Na^+-independent methionine and glucose uptake by isolated jejunal and ileal BBMV from pigs fed an *Aspergillus* product

Substrate	System	Parameter[a]	Diet				SE	pb	pc	Pd
			Control		Aspergillus					
			Jejunum	Ileum	Jejunum	Ileum				
Methionine	Na^+-dependent	K_m	1.08	.90	.88	.80	.10	.49	.54	.82
		V_{max}	.18	.17	.14	.16	.01	.38	.77	.53
	Na^+-independent	K_m	1.07	1.15	1.14	1.29	.07	.90	.22	.45
		V_{max}	.23	.24	.28	.29	.02	.19	.59	.97
Glucose	Na^+-dependent	K_m	1.14	1.17	1.21	1.41	.05	.15	.27	.39
		V_{max}	.30	.28	.35	.35	.03	.33	.90	.99
	Na^+-independent	K_m	7.73	7.18	7.10	9.95	1.16	.67	.65	.50
		V_{max}	1.33	2.12	.85	1.46	.22	.34	.14	.69

[a] K_m and V_{max} are expressed as millimoles and nanomoles. mg protein^{-1}.sec^{-1}, respectively.
[b] Probability that a diet difference this large or larger could have occurred by chance.
[c] Probability that a site difference this large or larger could have occurred by chance.
[d] Probability that the diet*site interaction that was observed could have occurred by chance.

studies with rats, guinea pigs, and humans. The upper to middle jejunum in species such as rats, guinea pigs, and humans is usually recognized to have the greater potential glucose transport capability (Gray, 1981; Karasov et al., 1983; Harig et al., 1989). The results from our studies and those of Collington et al., (1990) indicate that sucrase activity in the ileum is greater than that of the jejunum in growing pigs. Therefore, these results suggest that the ileum of pigs plays a very important role in the absorption of glucose at the same or greater extent compared with the jejunum. In suckling pigs, the proximal intestine possessed almost twice the capacity of glucose transport as did the distal intestine (Vega et al., 1992). Collington et al., (1990) reported that the highest sucrase activity was observed in the proximal intestine of 7-d-old piglets and shifted to the distal intestine after weaning.

The relative proportion of total uptake of methionine attributable to the different modes of methionine transport varied with substrate concentrations. Because uptake by diffusion is dependent on a concentration gradient and because the process is not saturable, the relative amount of methionine uptake occurring by diffusion would be expected be increase. This is precisely what did occur in BBMV isolated from pigs and this was not influenced by diet or intestinal segment (Figure 5; Appendix Table 17).

Both Na^+-dependent and the Na^+-independent uptake of methionine are saturable, carrier mediated process, therefore, it would be expected that the relative amount of total methionine uptake occurring by these processes would decrease, especially at higher methionine concentrations. The data presented in Figure 5 and Appendix Table 17 show that this is what happened in the present study with pigs. Throughout the range of substrate concentrations measured, Na^+-

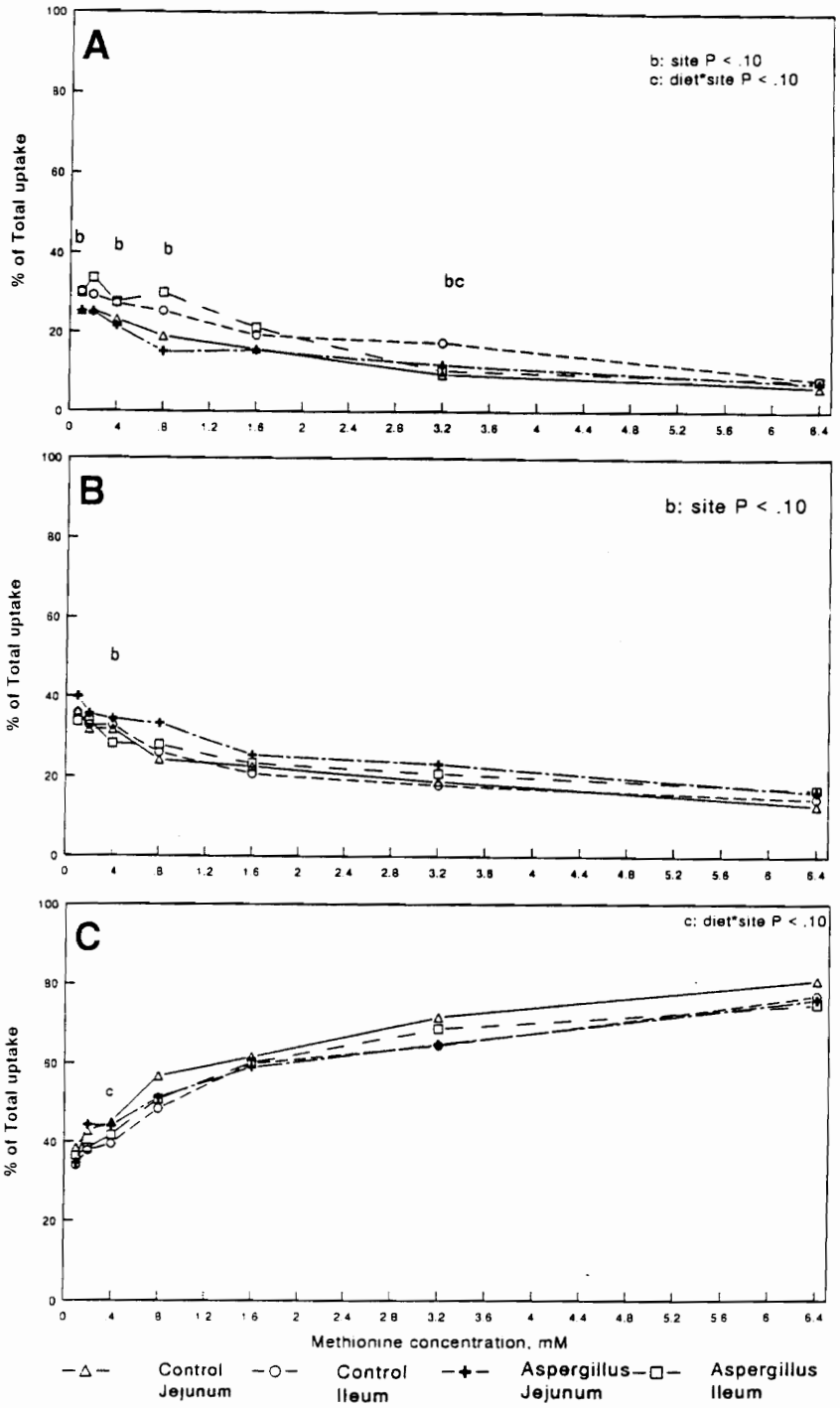

Figure 5. Relative contributions of Na^+-dependent (A), Na^+-independent (B), and diffusion (C) systems to total methionine uptake by jejunal and ileal BBMV from pigs fed a control diet and the control diet supplemented with an *Aspergillus* product. All data values are means from six animals. The SE for concentrations .1, .2, .4, .8, 1.6, 3.2, and 6.4 mM were 1.2, 1.4, 1.5, 1.2, 1.1, .7, .9 for Na^+-dependent uptake, 1.0, 1.1, 1.1, 1.7, 1.5, 1.5, 1.6 for Na^+-independent uptake, and .8, .9, 1.2, 1.3, 1.6, 1.2, % for diffusion, respectively.

dependent methionine uptake accounted for the lowest proportion of total uptake. Diet did not influence the relative uptake of methionine by Na^+-dependent or Na^+-independent uptake systems. There was a strong tendency for Na^+-dependent uptake to be relatively more important in ileal BBMV than in jejunal BBMV.

As with methionine, diffusion accounted for an increasingly greater proportion of total glucose uptake by BBMV as substrate concentration increased (Figure 6; Appendix Table 19). Neither diet nor intestinal site influenced the relative proportion of glucose uptake via diffusion. The relative uptake of glucose via Na^+-dependent carriers was greatest at low substrate concentrations and decreased as substrate concentration increased (Figure 6; Appendix Table 19). After a slight increase up to a substrate concentration of 1.2 mM, the relative amount of glucose uptake that occurred via Na^+-independent carriers remained relatively constant regardless of substrate concentration. Like the other modes of glucose uptake, neither diet nor intestinal site influenced the relative uptake of glucose by BBMV isolated from pig small intestine.

Malo (1991) investigated the relative contribution of each mode of uptake with human jejunal and ileal BBMV at a .5 mM methionine concentration. He found that the uptake by Na^+-dependent, Na^+-independent, and diffusion systems to be 19.0, 9.0, and 72% of total jejunal methionine uptake and 17, 15, and 68% of total ileal methionine uptake. Wolfram et al. (1986) observed that saturable transport components predominated up to 5 mM of D-glucose concentration. However, above 5 mM, the diffusive system was more important in the intestinal BBMV of pigs Those results are similar to our observations of methionine and glucose uptake. Generally, at low concentrations of substrate, methionine uptake via both diffusion and carrier mediated systems seemed to be important, while

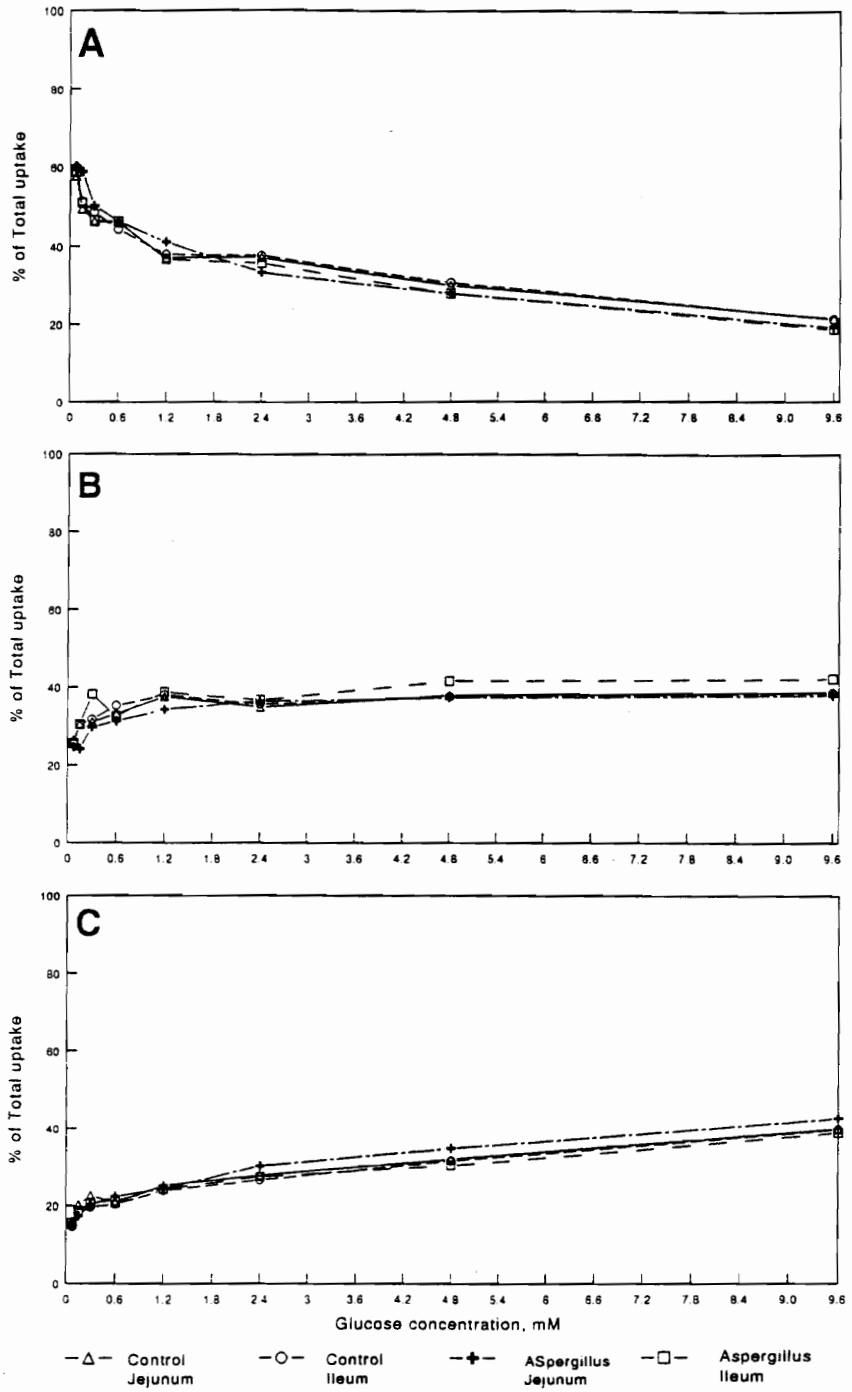

Figure 6. Relative contributions of Na^+-dependent (A), Na^+-independent (B), and diffusion (C) systems to total glucose uptake by jejunal and ileal BBMV from pigs fed a control diet and the control diet supplemented with an *Aspergillus* product. All data presented are means from six animals. The SE for concentrations .75, .15, .3, .6, 1.2, 2.4, 4.8, and 9.6 mM were 2.4, 2.4, 2.3, 1.6, 1.7, 1.7, 1.3, 1.5 Na^+-dependent uptake, 1.2, 1.4, 2.0, 1.1, 1.4, 1.4, 1.0, 1.1 for Na^+-independent uptake, and .7, .8, .7, .7, .9, .9, .8, 1.1 % for diffusion, respectively.

uptake via carrier mediated systems is predominantly responsible for glucose uptake.

Experiment 2. Total uptake of methionine by isolated BBMV was not influenced by the inclusion of the *Aspergillus* product in the diet (Figure 7; Appendix Table 20). Likewise, neither Na^+-dependent uptake nor diffusion were influenced by the feeding of the *Aspergillus* product (Figures 7 and 8; Appendix Table 20). Uptake of methionine by Na^+-independent carriers was numerically lower at all substrate concentrations when the *Aspergillus* product was fed (Figure 8; Appendix Table 20). While the difference was apparent across all concentrations, the difference was significant ($P < .10$) only at three of the seven substrate concentrations.

There appeared to be some influence of intestinal site on the uptake of methionine (Figures 7 and 8; Appendix Table 20). At some substrate concentrations, total uptake of methionine was greater ($P < .08$) in ileal BBMV than in jejunal BBMV. Within BBMV isolated from lambs fed the *Aspergillus* product, total uptake of methionine was always numerically greater for the ileum. This same trend was generally true for each of the three components of methionine uptake that contribute to total uptake. Conversely, uptake of methionine by BBMV from both jejunal and ileal tissue from fed lambs fed the control diet was very similar regardless of mode of uptake.

The kinetic data are presented in Table 14. Neither diet nor site influenced the K_m or V_{max} for Na^+-dependent methionine transport. For Na^+-independent transport, ileal BBMV exhibited a higher ($P < .10$) K_m than did jejunal BBMV. The rate of transport, V_{max}, was not influenced. The K_m of the Na^+-independent

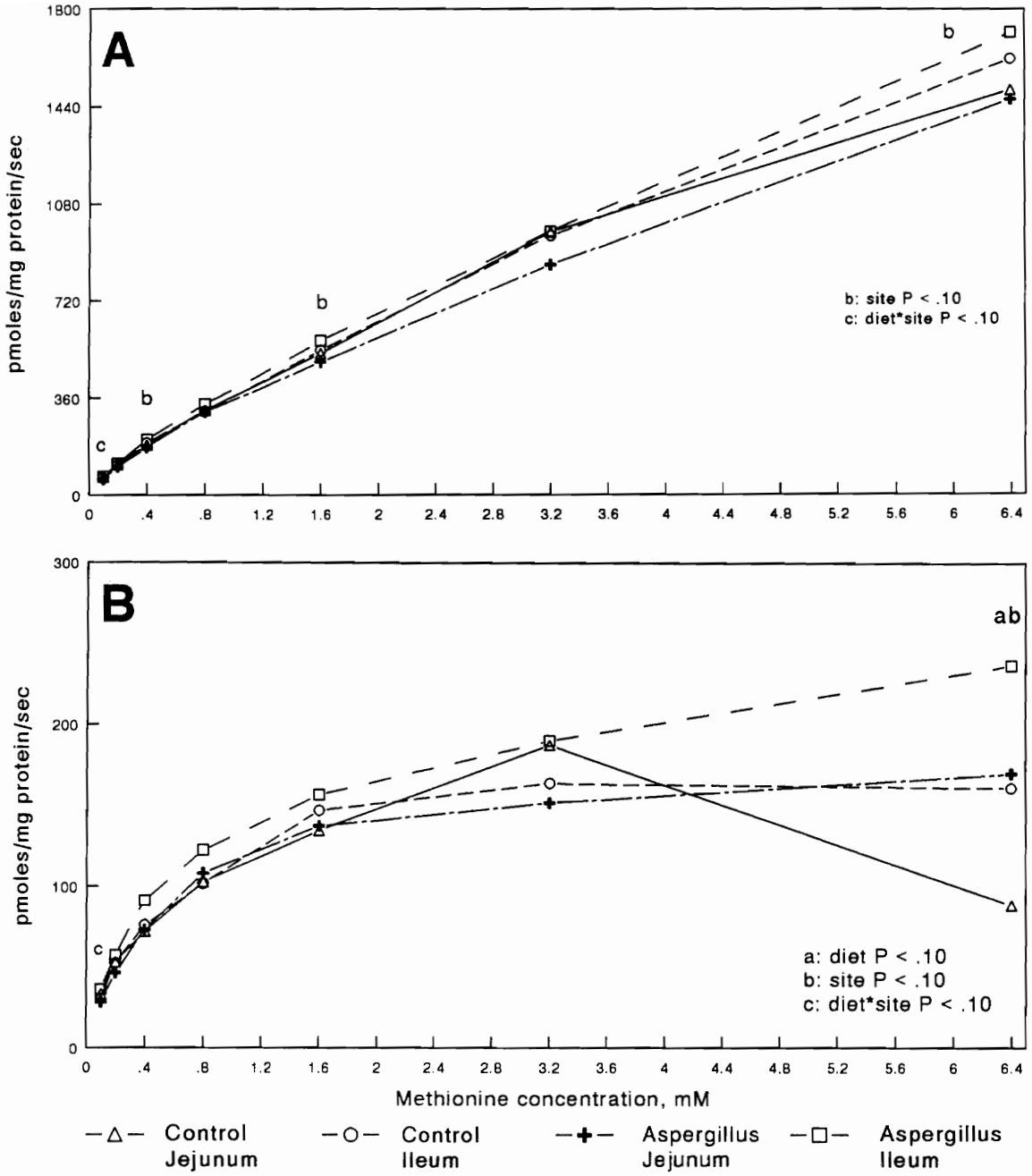

Figure 7. Total uptake (A) and Na^+-dependent uptake (B) of methionine by jejunal and ileal BBMV from lambs fed a control diet and the control diet supplemented with an *Aspergillus* product. All data values are means from six animals. The SE for concentrations of .1, .2, .4, .8, 1.6, 3.2, and 6.4 mM were 1.7, 2.8, 4.6, 7.2, 11.0, 26.0, 38.5 and 1.5, 2.8, 3.5, 5.2, 8.4, 12.9, 13.1 pmoles/mg protein/sec for total uptake and Na^+-dependent uptake, respectively.

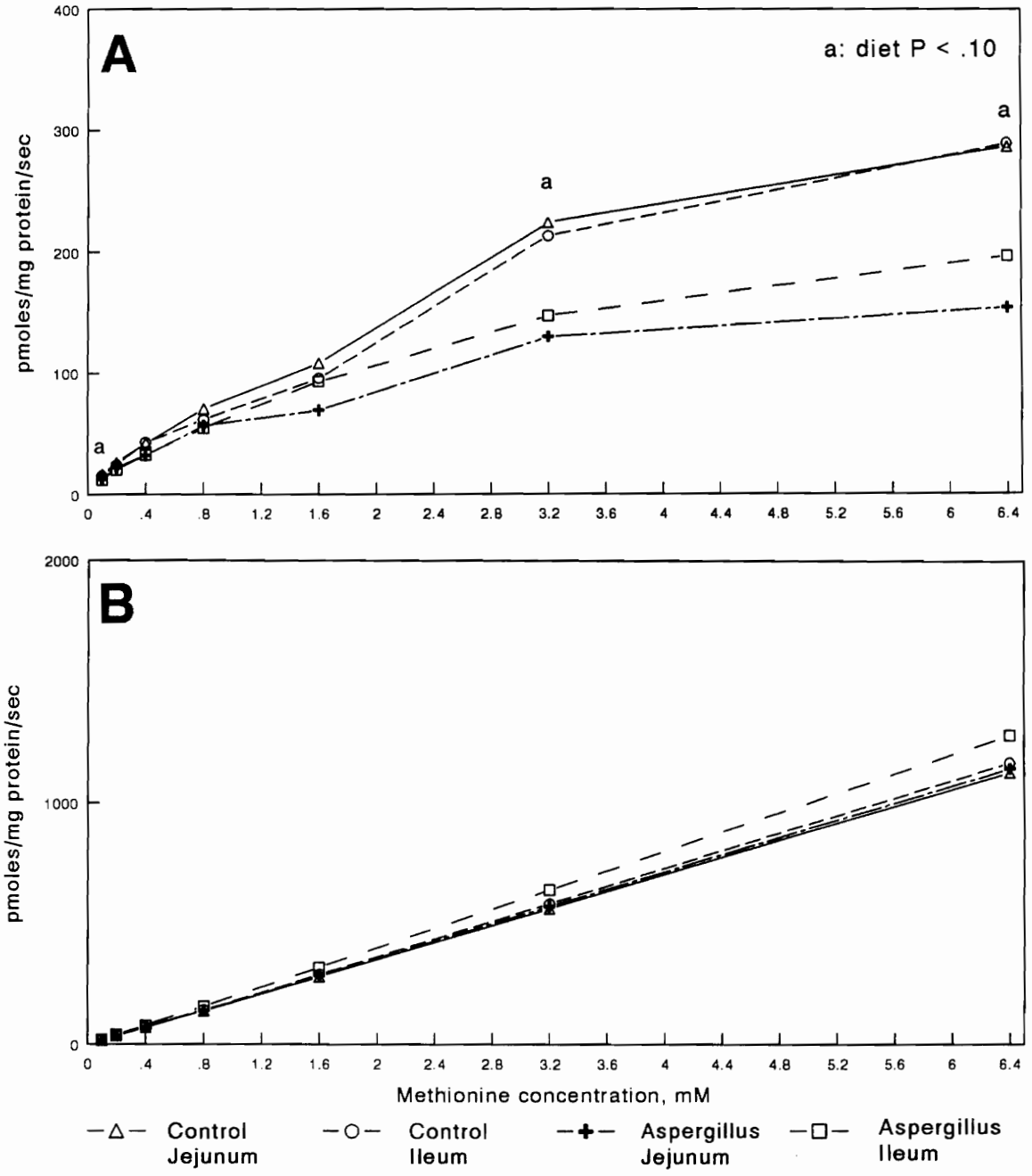

Figure 8. Na^+-independent uptake (A) and diffusion (B) of methionine by jejunal and ileal BBMV from lambs fed a control diet and the control diet supplemented with an *Aspergillus* product. All data values are means from six animals. The SE for concentrations of .1, .2, .4, .8, 1.6, 3.2, and 6.4 mM were 1.0, 1.8, 3.4, 5.1, 10.0, 20.7, 24.2 and .6, 1.2, 2.5, 4.9, 9.9, 19.8, 39.6 pmoles/mg protein/sec for Na^+-independent uptake and diffusion, respectively.

transport of methionine was about double the Km of Na^+-dependent transport indicating that the Na^+-independent transporters had only about one-half the affinity for methionine. Both systems had similar maximal capacities.

In comparision to lambs fed the control diet, total uptake of glucose by isolated jejunal and ileal BBMV from lambs fed the *Aspergillus* product was lower (Figure 9; Appendix Table 22). Except for the two higher substrate concentrations, these difference were either significant ($P < .08$) or approached significance ($P < .16$). This same general tendency was observed in Na^+-dependent uptake, Na^+-independent uptake, and diffusion (Figures 9 and 10; Appendix Table 22). The difference is most notable with Na^+-dependent uptake (Figure 9).

Glucose uptake was strongly influenced by intestinal location. Total uptake of glucose by jejunal BBMV far exceeded ($P < .04$) total uptake by ileal BBMV (Figure 9; Appendix Table 22). This was mostly due to the Na^+-dependent glucose component (Figure 9; Appendix Table 22). Sodium-dependent glucose uptake by jejunal BBMV averaged about 2.7 times the Na^+-dependent uptake by ileal BBMV. Also, total and Na^+-dependent uptake by jejunal BBMV from lambs fed the control diet was greater than by lambs fed the diet containing the *Aspergillus* product at several concentrations, the diet*site interactions are significant or approached significance. While the magnitude was less, diffusion also tended ($P < .11$) to be greater in the jejunal BBMV. Sodium-independent uptake was similar between intestinal sites.

The kinetic data presented in Table 14 support these observations. The V_{max} for Na^+-dependent glucose uptake was greater in BBMV from lambs fed the control diet ($P < .03$) and in jejunal BBMV ($P < .002$). The K_m for Na^+-dependent glucose uptake also was lower ($P < .005$) for jejunal BBMV compared

Table 14. The K_m and V_{max} of Na^+-dependent and Na^+-independent methionine and glucose uptake by isolated jejunal and ileal BBMV from lambs fed an *Aspergillus* product

Substrate	System	Parameter[a]	Diet				SE	pb	pc	pd
			Control		Aspergillus					
			Jejunum	Ileum	Jejunum	Ileum				
Methionine	Na^+-dependent	K_m	.53	.57	.58	.56	.002	.65	.73	.52
		V_{max}	.18	.19	.18	.21	.006	.41	.15	.21
	Na^+-independent	K_m	1.07	1.31	1.13	1.35	.06	.70	.10	.93
		V_{max}	.19	.20	.14	.18	.01	.14	.20	.56
Glucose	Na^+-dependent	K_m	.12	.14	.12	.16	.002	.23	.005	.23
		V_{max}	.24	.12	.17	.08	.01	.03	.002	.43
	Na^+-independent	K_m	2.88	1.59	2.59	1.79	.11	.85	.006	.32
		V_{max}	.49	.30	.30	.29	.03	.36	.05	.46

[a] K_m and V_{max} are expressed as millimoles and nanomoles.mg protein^{-1}.sec^{-1}, respectively.
[b] probability that a diet difference this large or larger could have occurred by chance.
[c] probability that a site difference this large or larger could have occurred by chance.
[d] Probability that the diet*site interaction that was observed could have occurred by chance.

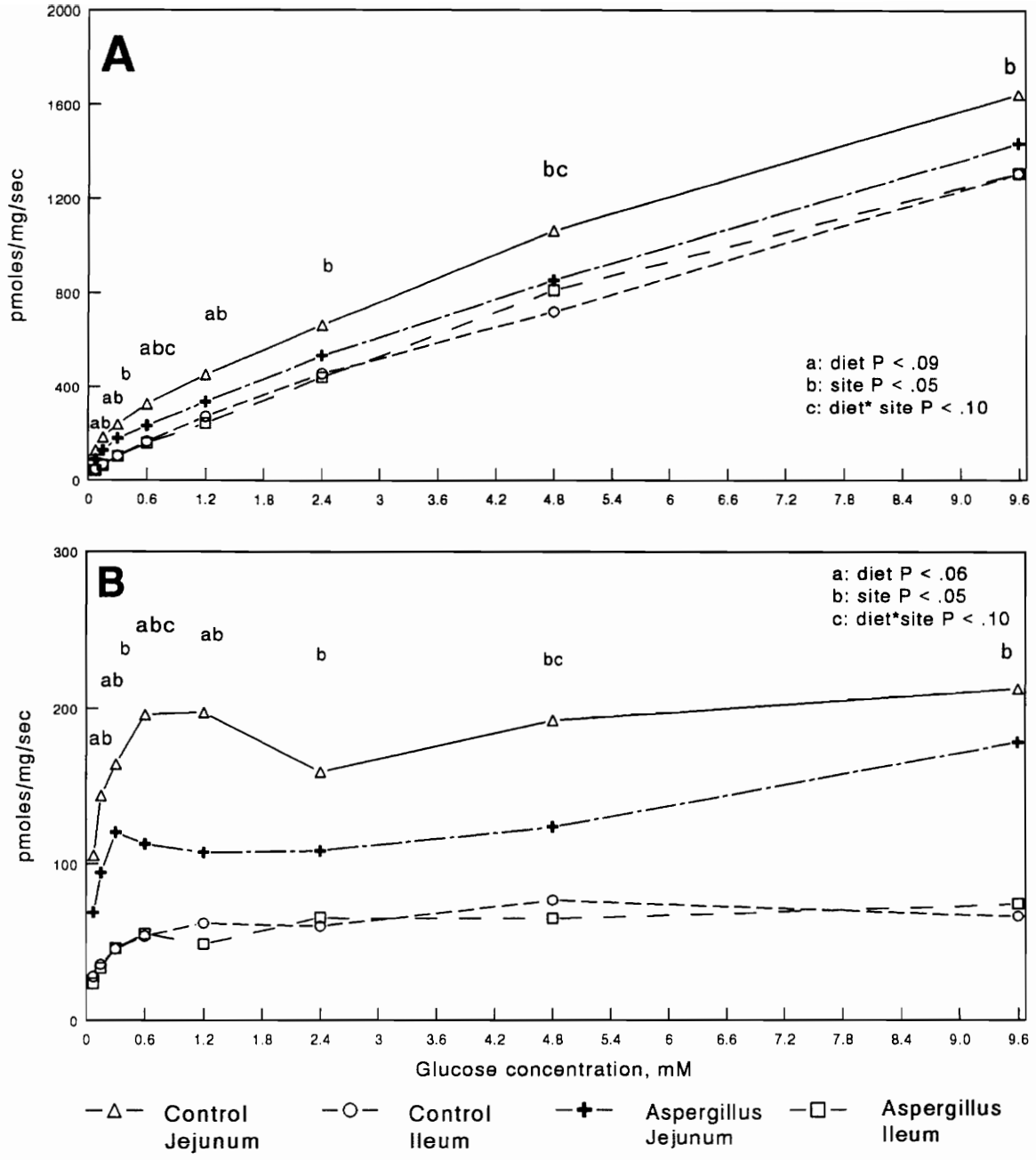

Figure 9. Total uptake (A) and Na^+-dependent uptake (B) of glucose by jejunal and ileal BBMV from lambs fed a control diet and the control diet supplemented with an *Aspergillus* product. All data values are means from six animals. The SE for concentrations of .075, .15, .3, .6, 1.2, 2.4, 4.8, and 9.6 mM were 5.3, 7.8, 4.1, 12.3, 15.1, 23.5, 41.7, 66.6 and 4.6, 7.2, 9.0, 12.3, 15.1, 23.5, 17.8, 17.3 pmoles/mg protein/sec for total uptake and Na^+-dependent uptake, respectively.

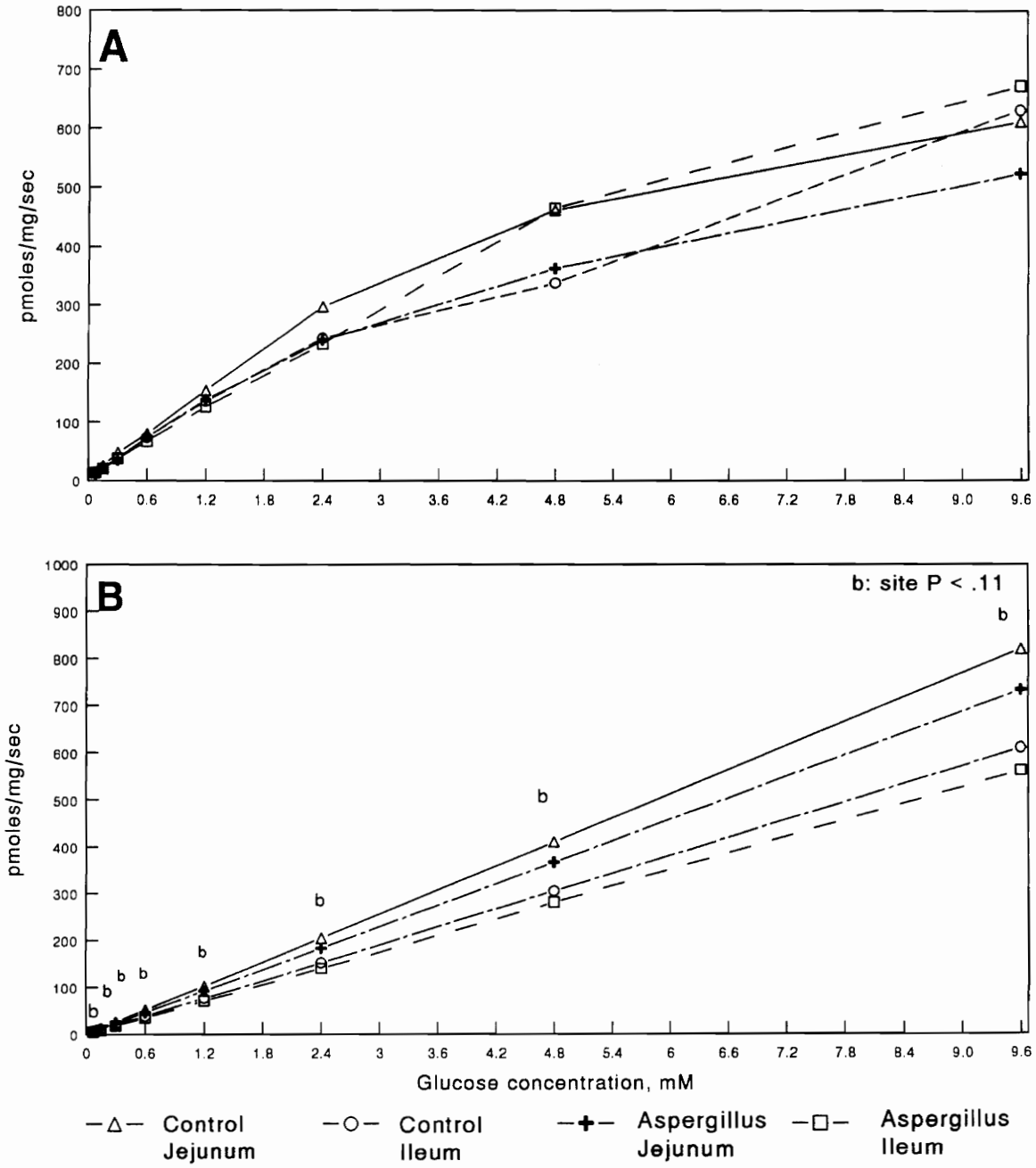

Figure 10. Na^+-independent uptake (A) and diffusion (B) of glucose by jejunal and ileal BBMV from lambs fed a control diet and the control diet supplemented with an *Aspergillus* product. All data values are means from six animals. The SE for concentrations of .075, .15, .3, .6, 1.2, 2.4, 4.8, and 9.6 mM were 1.0, 1.9, 2.9, 5.7, 10.9, 18.9, 42.5, 64.8 and .4, .9, 1.7, 3.4, 6.9, 13.7, 27.4, 54.2 pmoles/mg protein/sec for Na^+-independent uptake and diffusion, respectively.

with ileal BBMV. Diet had essentially no effect on K_m or V_{max} of Na^+-independent glucose transport. The K_m and V_{max} of Na^+-independent uptake were both higher ($P < .006$ and $P < .05$, respectively) for jejunal BBMV. The K_m of the Na^+-independent transport of glucose was approximately 10- to 20-fold higher than that of Na^+-dependent transport indicating that the Na^+-independent transporters had only about one-tenth to one-twentieth the affinity for glucose. Also the Na^+-independent system of glucose uptake possessed about two-fold greater maximal capacities.

We are not aware of any studies that have been conducted to determine whether fungal or yeast products affect uptake of nutrients in the small intestine, although studies have been conducted to evaluate the influence of *Aspergillus* on the apparent digestibility of nutrients in the rumen and total gastrointestinal tract. Several studies reported that digestibility of CP, OM, or fiber components are improved in the rumen or total tract with the feeding of *Aspergillus* products (Arambel et al., 1987; Wiedmeier et al., 1987; Gomez-Alarcon et al., 1990). However, other studies have failed to demonstrate that *Aspergillus* products improve the digestibility of these components (Oellermann et al., 1990; Denigan et al., 1992). Interestingly, in contrast to cows fed a control diet, the duodenum of cows fed an *Aspergillus* product possessed feed particles containing a dense population of fungi similar to normal rumen fungi under microscopic examination (Prange et al., 1979). Nisbet and Martin (1990) reported that *Selenomonas ruminatium*, which is a major cellulolytic bacterium in the rumen, exhibited a remarkably enhanced lactate uptake in the presence of an *Aspergillus* product.

Our results indicated that the supplementation of an *Aspergillus* product did not induce the improvement of any parameters of methionine uptake by isolated

BBMV from lambs. However, with glucose uptake, total and Na^+-dependent glucose uptake were decreased significantly in response to the *Aspergillus* product. Caton et al. (1993) indicated that duodenal flow of total, essential, and nonessential amino acids tended to be increased ($P < .10$) by the inclusion of *Aspergillus* products to steers. Meanwhile, Williams et al. (1990) reported that diets fortified with yeast gave rise to a significant reduction (3-fold) of hexose-unit oligosaccharide in the rumen of steers. According to Martin and Nisbet (1990), soluble-starch fermentation tended to increase production of H_2, CH_4, and VFA when mixed ruminal microorganisms were incubated with *Aspergillus* product. Several researchers observed increased numbers of ruminal bacteria and greater ruminal fermentation activity when cows were fed a diet supplemented with *Aspergillus* products (Wiedmeier et al., 1987; Frumholtz et al., 1989; Beharka et al., 1991).

These fungal and yeast products can produce amylase and amyloglucosidase to aid the digestion of starch to glucose directly (French, 1973; Ustyazhanina et al., 1984). Also, *Aspergillus* products are capable of partial depolymerization of structural cellulose, although enzymes produced by fungi do not depolymerize cellulose completely to glucose (Boing, 1983; Mountfort, 1987).

Presumably, *Aspergillus* products do not directly influence the transport capacity of methionine or glucose by the brush border of the small intestine. It seems that the increased degradation of starch by the inclusion of *Aspergillus* products may indirectly affect the intestinal glucose transport capacity. Generally, elevated protein (amino acid or peptide) or carbohydrate (glucose) level in diets resulted in a stimulated amino acid or glucose active transport as a result of increased maximal capacity (V_{max}) of intestinal transporters compared to low

concentrations of protein or glucose, respectively (Ferraris et al., 1989, 1992b). In our experiment, reduced total and Na^+-dependent glucose uptake in response to the *Aspergillus* product may be due to an increase in starch (carbohydrate) degradation in the rumen and less starch passing to the small intestine. Shirazi-Beechey et al., (1991a) pointed out that the infusion of 30 mM of D-glucose into the proximal intestine of adult sheep resulted in a 40- to 80-fold greater Na^+-dependent glucose transport when compared to the same age of sheep fed grasses. This result suggested that intact glucose may affect the transcription or translation of genes expressing Na^+-dependent glucose transport protein (Shirazi-Beechey., 1991a). Kreikemeier et al. (1991) observed that increased abomasal infusion of glucose, starch, or dextrin caused a continual increase in net glucose absorption across the small intestine.

In ruminants, much of the dietary carbohydrate may be fermented to VFA by ruminal microorganisms, and only small amounts of hexoses may pass into the small intestine under normal feeding conditions (Weekes, 1979). However, in our experiment, the lambs were provided an 85% cracked corn-soy diets with 15% ground orchard grass hay. Probably, in this kind of feeding regimen, a considerable amount of starch may escape from the rumen to the small intestine. Owens et al. (1986) reported that 18 to 42% of dietary starch reached the small intestine and 42 to 88% of the starch reaching the small intestine was digested, based upon a review of 42 trials with cattle fed high-grain diets. Janes et al. (1985) reported that net glucose absorption across the small intestine could account for the disappearance of starch in the small intestine.

In contrast to monogastric animals such as rats, monkeys, pigs, and humans, the ruminant ileum is more active than the jejunum for amino acid absorption

(Johns and Bergen, 1973; Phillips et al., 1979; Guerino and Baumrucker, 1987). In sheep, it was shown that the ileum possessed a greater absorption capacity for threonine than the ileum (Phillips et al., 1976). According to Wilson and Webb (1990a), ileal BBMV from bovine had greater total methionine uptake compared with jejunal BBMV. Data from the present study indicated that total methionine uptake by ileal BBMV was greater than jejunal BBMV. As with ruminants, it also has been shown that the distal ileum of the rabbit has the maximal rate of transport along the entire intestine (Munck, 1985; Munck and Munck, 1992a). Conceivably, species differences are an important factor related to intestinal site of amino acid uptake. The range of pH of 7 to 8 is the optimal level for intestinal protease ability, but the contents of the small intestine of ruminants approach this range near the last one-half to two-thirds of the intestine (Ben-Ghedalia et al., 1974). Thus, the luminal milieu in ruminants may be less compatible with maximal proteolytic activity and transport capability of amino acids in the middle to upper portions of the small intestine (Webb, 1992).

Regarding the regional comparison of the intestine for glucose uptake, there were substantial difference across sites (Figure 9). Total glucose uptake by the jejunal BBMV exceeded that of the ileal BBMV by 2.7-fold. This clearly indicates that the jejunum of lambs is the site of maximal glucose transport capacity. This is similar to other species such as rats, guinea pigs, and humans. Russell et al. (1981) reported that the specific activity of maltase in cattle was highest in the jejunum and decreased toward the ileum. Presumably, the quantitatively small amount of luminal glucose flowing past the ileum accounts for the low capacity of glucose transport compared with the jejunum (Harig et al., 1989; Ferraris et al., 1990).

The importance of the relative contributions of the Na^+-dependent, Na^+-independent, and diffusion pathways to total uptake of methionine and glucose at various substrate concentrations is illustrated in Figures 11 to 12 and Appendix Tables 21 and 23. Diffusion provided a significant contribution for methionine uptake by the jejunum and ileum. At substrate concentrations above .4 mM, uptake via diffusion accounted for the highest portion of total uptake. The Na^+-dependent uptake was the dominant pathway for methionine uptake at methionine concentrations below .4 mM. As substrate concentration was increased, this system became of less relative importance. Overall, Na^+-independent uptake was the least important contributor.

Moe et al. (1987) observed that the relative portion of Na^+-dependent, Na^+-independent, and diffusion uptake accounted for 14, 37, and 49% of total methionine uptake when methionine concentration was .1 mM in bovine ileal BBMV. Wilson and Webb (1990a) using the bovine found that methionine uptake by Na^+-dependent, Na^+-independent, and diffusion systems to be 53, 19, and 28% of total uptake at .125 mM methionine and 28, 16, and 56% of total uptake at 1.25 mM methionine. Likewise, Karasov et al. (1986) noted that the Na^+-dependent pathways for methionine uptake were the most prevalent pathways when relative portion of uptake was measured at a .01 mM methionine concentration.

As expected, the data (Figure 12 and Appendix Table 23) revealed that diffusion of glucose became increasingly more important as substrate concentration increased. However, diffusion was not nearly so dominant a mode of uptake as it was with methionine. In fact, the two concentration-dependent forms of uptake, diffusion and Na^+-independent uptake, shared very similar magnitudes

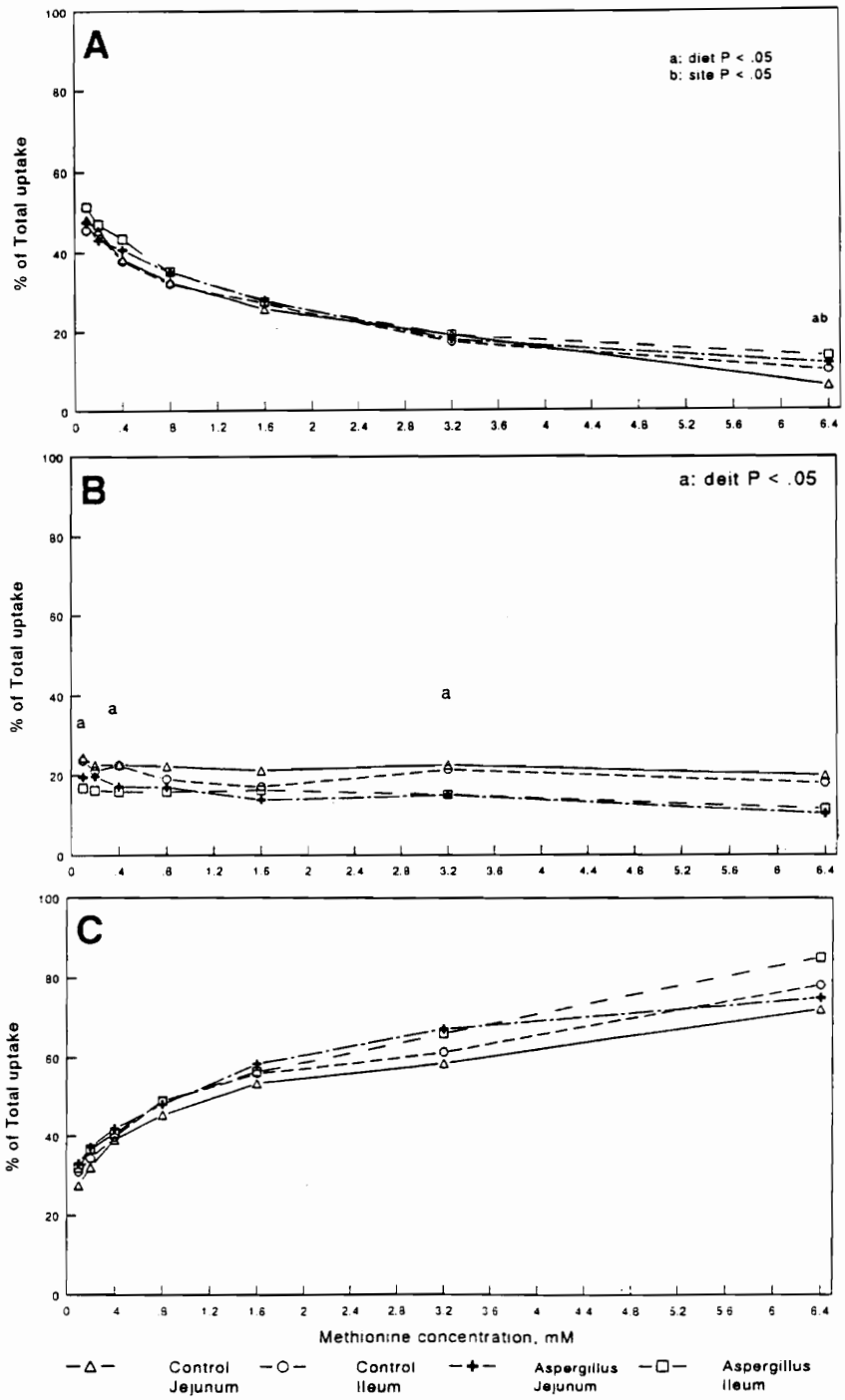

Figure 11. Relative contributions of Na^+-dependent (A), Na^+-independent (B), and diffusion (C) systems to total methionine uptake by jejunal and ileal BBMV from lambs fed a control diet and the control diet supplemented with an *Aspergillus* product. All data are means from six animals. The SE for concentrations .1, .2, .4, .8, 1.6, 3.2, and 6.4 mM were 1.4, 1.7, 1.2, 1.4, 1.3, 1.0, .7 or Na^+-dependent uptake, 1.5, 1.6, 1.8, 1.6, 1.8, 1.8, 1.6 for Na^+-independent uptake, and .9, 1.0, 1.2, 1.2, 1.5, 2.0, 1.8 % for diffusion, respectively.

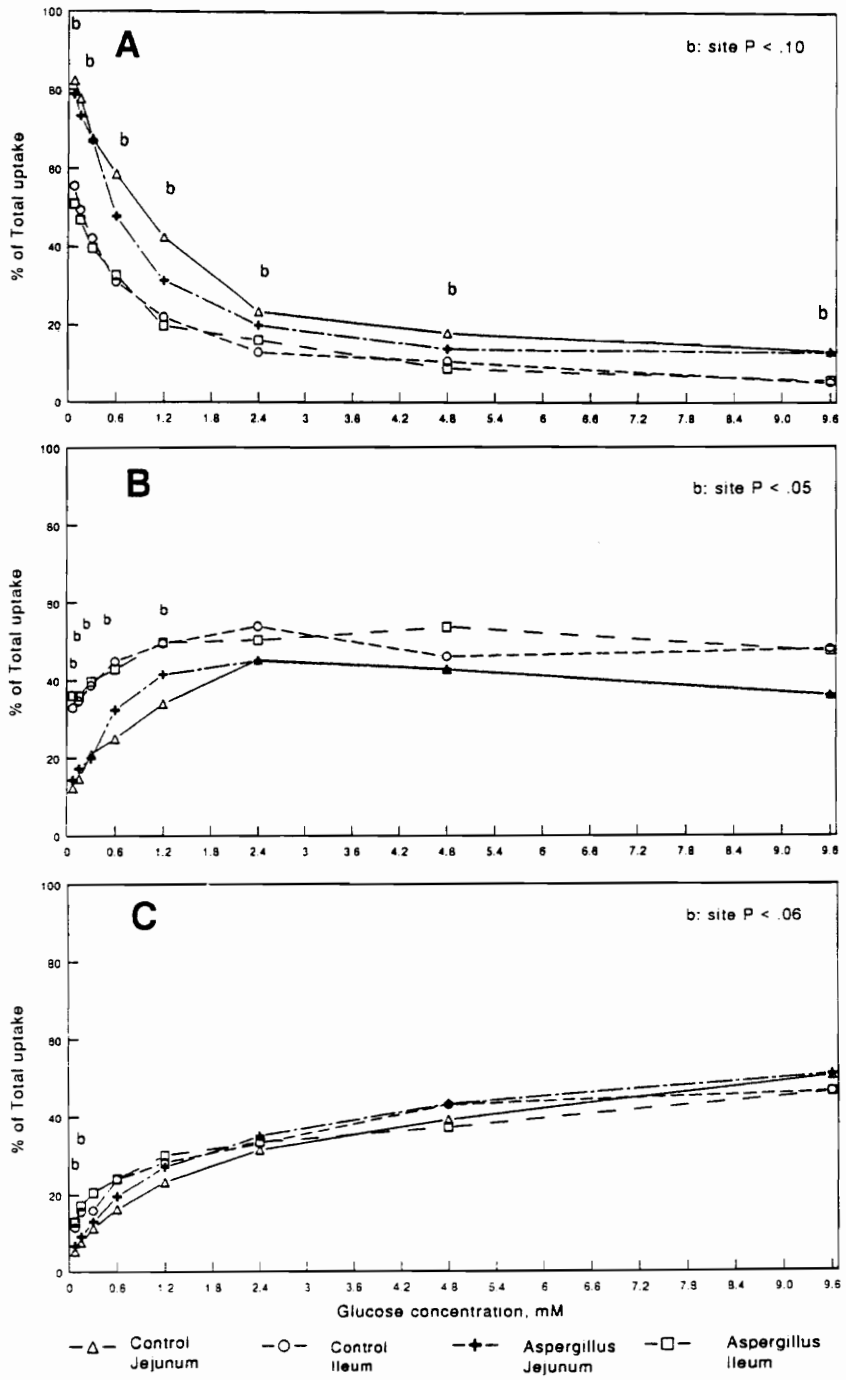

Figure 12. Relative contributions of Na^+-dependent (A), Na^+-independent (B), and diffusion (C) systems to total glucose uptake by jejunal and ileal BBMV from lambs a fed control diet and the control diet supplemented with an *Aspergillus* product. All data are means from six animals. The SE for concentrations of .075, .15, .3, .6, 1.2, 2.4, 4.8, and 9.6 mM were 2.4, 2.7, 2.6, 2.6, 1.9, 2.0, 1.7, 1.2 for Na^+-dependent uptake, 1.7, 2.3, 2.1, 2.3, 2.7, 2.8, 3.7, 3.6 for Na^+-independent uptake, and 1.2, 1.7, 1.9, 2.2, 2.4, 2.8, 3.4, 3.8 % for diffusion, respectively.

of contribution to total glucose uptake. Sodium-dependent glucose uptake was relatively more important at low glucose concentrations and relative glucose uptake by this mechanism declined rapidly as substrate concentration increased. Interestingly, the relative contribution of each mode of uptake to total uptake between the jejunum and ileum varied. It appeared that Na^+-dependent uptake made a much greater contribution to total uptake in the jejunal compared with ileal BBMV.

Concerning the relative importance of glucose uptake pathways in lamb intestinal BBMV, our data is very similar to that of Wolffram et al.(1986), which indicated that the saturable transport component predominated up to 5 mM of D-glucose. Stevens et al. (1984) also reported that the high affinity system was overwhelmed at low glucose concentrations, but the diffusive system is more important at concentrations above 2 mM. The conclusion that can be drawn is that active transport of substrate by intestinal BBMV from lambs is quantitatively the most important when methionine or glucose concentrations are low and diffusion makes progressively greater contributions to total uptake as substrate concentration increases.

Conclusions and Implications

Under the conditions of the present studies, the *Aspergillus* product did not alter growth or feed utilization in weanling pigs or growing, finishing lambs.

The results of characterization of isolated BBMV clearly demonstrated that purity of intestinal BBMV could be considered as adequate for the purpose of methionine and glucose transport experiments, because they generally display over

6- to 20-fold enrichments in the specific marker enzymes such as alkaline phosphatase or sucrase with little apparent contamination by other cellular organelles.

The inclusion of the *Aspergillus* product to the pigs diet did not induce changes in intestinal mucosal cell enzyme profile or transporter affinity or capacity of methionine and glucose transport. However, the supplementation of this product to the lamb diet decreased jejunal mucosal cell weight and the capacity for total and Na^+-dependent glucose transport, although neither intestinal enzyme profile nor methionine transport were altered. It appears that there is same correlation between mucosal weight and glucose transport capacity in isolated intestinal BBMV from lambs. Apparently, the jejunal BBMV showed greater adaptive response to the feeding of *Aspergillus* product compared with the ileal BBMV in lambs. In our experiment, reduced total and Na^+-dependent glucose uptake in response to the *Aspergillus* product may be due to an increase in starch (carbohydrate) degradation in the rumen and less starch passing to the small intestine.To test this hypothesis, the effect of *Aspergillus* on ruminal degradation of starch should be investigated. Furthermore, the association between the expression of glucose transport protein and ruminal and small intesinal digestion of carbohydrate (glucose) needs exploration.

There was the site difference for sucrase activity in pigs and alkaline phosphatase activity in lambs, respectively. Also, isolated jejunal BBMV from lambs possessed greater capacity for glucose transport compared with that of ileal BBMV. Diffusion of methionine made the predominat contribution to total methionine transport, while Na^+-dependent glucose transport played the most

important role in the relative contribution to total uptake of glucose transport under physiological conditions.

Chapter IV

General Discussion

As discussed in Chapter III, the supplementation of the *Aspergillus* product to a pig diet did not change either intestinal mucosal cell weight, enzyme profile, or transporter affinity and capacity for methionine and glucose. However, the feeding of the *Aspergillus* product to lambs gave rise to a decrease in jejunal mucosal weight and capacity for total and Na^+-dependent uptake of glucose, while neither intestinal enzyme profiles nor transport systems for methionine were altered. Therefore, it appears that the *Aspergillus* product, as a feed additive, may have more metabolic influence on the ruminant than on monogastric animals.

Feeding products based upon either fungi or yeasts to ruminants sometimes result in alterations in ruminal fermentation. The present observations of a decrease in jejunal mucosal cell weight and transport capacity for glucose by isolated BBMV from lambs may result from increased ruminal fermentation in response to feeding of the *Aspergillus* product. According to Williams et al. (1990), hexose-unit oligosaccharides containing maltose and maltriose in ruminal fluid of steers fed a 60:40 concentrate to forage diet were reduced 3-fold by the feeding of yeast cultures. Also, several studies reported that ruminal fermentation was increased in the presence of *Aspergillus* products. This may be brought about by the stabilization of the ruminal environment through reductions in ruminal lactate concentration and increases in ruminal pH which leads to increased ruminal bacteria and subsequently an increase in ruminal degradation of starch and structural carbohydrate (Williams et al., 1990). Presumably, the reduced glucose

transport in response to the *Aspergillus* product may be associated with the increased rumen degradation of starch when lambs were fed an 85% grain diet. Subsequently, the decreased post-ruminal supply of carbohydrate may result in a decreased expression of the small intestinal glucose transport protein (adaptive regulation). The decrease in total glucose uptake by isolated BBMV from lambs fed the *Aspergillus* product was largely due to a decreased Na^+-dependent uptake. The decreased intact sugar might induce less transcription or translation of the Na^+/glucose cotransporter gene in the enterocyte. The current belief is that most intestinal nutrient transporters are, at least to some extent, regulated by their substrates. In the case of nutrient transporters for sugars, amino acids and peptides, an increasing concentration of some these nutrients in the intestinal lumen will stimulate the development of increased transport capacity or rate. (Buddington and Diamond, 1989; Ferraris and Diamond,1989).

Alterations of ruminal fermentation such as VFA production and ratio of acetate to propionate in the presence of *Aspergillus* products should not be overlooked as factors that may effect digestion (uptake) or metabolism in the small intestine. It may be possible that one or more of the end products of carbohydrate fermentation in some way regulate mucosal development and glucose transport capacity in the small intestine. With increased fermentation, more end products (i.e. VFA) may escape absorption from the stomach and pass into the small intestine. At this point they may be absorbed by the enterocytes and function as down-regulation for mucosal cell development and (or) glucose transport. Evidence for such a role of VFA, however, is not available.

Another possible factor that may influence glucose transport in response to the *Aspergillus* product is the alteration of mucosal cell maturation or cell

migration rate and expression of digestive enzymes such as amylase and maltase. In the present study, feeding of the *Aspergillus* product caused a reduction of jejunal mucosal weight (14.9%) of lambs. We did not monitor morphological or histological changes or digestive enzymes. Nevertheless, the possibility of this alteration in response to the *Aspergillus* product can not be exclude. Often, improvement of growth of pigs may be due to changes in gastrointestinal tract development (Collington et al., 1988). It is apparent that changes in nutrient metabolism or modulations of mucosal cell function of the intestine can have a major influence on the pattern of transport capacity of the small intestine.

Other feasible factors that may affect the expression of glucose uptake by the feeding of *Aspergillus* product include changes in rate of flow of digesta, gastrointestinal pH, and direct impact on mucosal cell transport properties.

As discussed, *Aspergillus* products may have their most direct effect on ruminal fermentation rather than on small intestinal nutrient transport. *Aspergillus* products may alleviate negative effects such as the difficulty associated with the digestion of structural carbohydrate, accumulation of lactate, and subsequent decreases in the number of cellulolytic bacteria in the rumen when animals are fed a high grain diet to enhance energy density under intensive management programs. However, if more starch is degraded in the rumen by the feeding of *Aspergillus* products, the efficiency of energy utilization will be decreased. Density of energy is much greater if carbohydrate is directly absorbed from the small intestine rather than being fermented in the rumen. Digestion of starch in the small intestine resulted in a 42% improvement of energy density compared with degradation of starch in the rumen (Owens et al., 1985). If our assumption about less carbohydrate passing through the small intestine by the feeding of *Aspergillus*

product is true, one might question what kind of a feeding regimen is the most beneficial for animal nutrition when using *Aspergillus* products. It seems that the inclusion of the *Aspergillus* product in a high-grain feeding regimen may not always be beneficial for livestock production systems in terms of energy efficiency. Further research of these interactions between supplementation of *Aspergillus* products and level of concentrate in the diet is necessary to optimize the efficiency of animal production system.

Also, it may be argued as to how much non-structural carbohydrate present in high grain diets can pass to the small intestine without ruminal degradation and how much the amylase and amylogucosidase *Aspergillus* product contains to aid the hydrolysis of alpha-linked glucose polymers (starch) in the rumen.

To prove the efficacy of *Aspergillus* on the expression of intestinal glucose transporters, study of the association between the expression of transporters and ruminal and small intestinal digestion of starch is necessary. Because, in ruminants, the glucose transport mechanisms involved in the regulation of dietary substrate are poorly understood compared with monogastric animals which utilize glucose as the major substrate for energy storage and oxidation, studies should be undertaken to further our knowledge in this area.

Literature Cited

Adibi, S. A., and S. J. Gary. 1967. Intestinal absorption of essential amino acids in man. Gastroenterology. 52:837-845.

Adibi, S. A., and Y. S. Kim. 1981. Peptide absorption and hydrolysis. In: L. R. Johnson, (Ed.) Physiology of the Gastrointestinal Tract. pp 1073-1095. Raven Press, New York.

Allison, M. J. 1989. Microbiology of the rumen and small and large intestines. In: M. J. Swenson (2nd Ed.) Duke's Physiology of Domestic Animals. pp 340-350. Cornell University Press, Ithaca, New York.

Allison, M. J., and M. P. Bryant. 1963. Biosynthesis of branched-chain fatty acids by rumen bacteria. Arch. Biochim. Biophys. 101:269-277.

Argenzio, R. A. 1989. Digestive absorption of carbohydrate, fat, and protein. In: M. J. Swenson (2nd Ed.) Duke's Physiology of Domestic Animals. pp 301-310. Cornell University Press, Ithaca, New York.

Arambel, M. J., R. D. Wiemeiser, and J. L. Walters. 1987. Influence of donor animal adaptation to added yeast culture and/or *Aspergillus oryzae* fermentation extract on in vitro rumen fermentation. Nutr. Rep. Int. 35:433-436.

Argiles, J. M., and F. J. Lopez-Soriano. 1990. Intestinal amino acid transport: An overview. Int. J. Biochem. 22(9):931-937.

Baker, R. D., and M. J. George. 1971. Pattern of neutral amino acid uptake along rat small intestine. Biochim. Biophys. Acta 225:315-325.

Beechey, R. B., R. B. Kemp, S. P. Shirazi-Beechey. 1987. Changes in the intestinal transport activities during the maturation of sheep. J. Physiol. 396:27p

Beharka, A. A., T. G. Nagaraja, and J. L. Morrill. 1991. Performance and ruminal function development of young calves fed diets with *Aspergillus oryzae* fermentation extract. J. Dairy Sci. 74:4326-4336.

Ben-Ghedalia, D., H. Tagari, A. Bondi, and A. Tadmor. 1974. protein digestion in the small intestine of sheep. Br. J. Nutr. 31:125-142.

Berteloot, A. 1984. Characteristics of glutamic acid transport by rabbit intestinal brush border membrane vesicles. Effect of Na^+, K^+, and H^+ gradients. Biochim. Biophys. Acta. 775:129-140.

Bodart, C. 1975. as cited by Chesson. Supplementary enzymes to improve the utilization of pigs and poultry diets. In: Recent advanced nutrition. Rowett Research Institute, Aberdeen, UK. Butterworths, London. pp 71-89.

Boing, J. T. P. 1983. Enzyme production. In: G. Reed (4thEd.) Industrial Microbiology. AVI Publishing Co., Inc. Westpoint, CT.

Brasitus, T. A., P. K. Dueja, M. J. G. Bolt, M. D. Sitrin, and C. Baum. 1989. Dietary triglycerol modulates sodium-dependent D-glucose transport, fluidity and fatty acid composition of rat small intestinal brush border membrane. Biochim. Biophys. Acta. 979:177-186.

Brot-Laroche, E., M. A. Serrano, B. Delhomme, and F. Alvarado. 1986. Temperature sensitivity and substrate specificity of two distinct Na^+-activated D-glucose transport systems in guinea pig jejunal brush border membrane vesicles. J. Biol. Chem. 261:6168-6176.

Bryant, M. P. 1973. Nutritional requirements of the predominant rumen celluloytic bacteria. Fed. Proc. 32:1809-1813.

Buddington, R, K., J. W. Chen, and J. M. Diamond. 1991. Dietary regulation of intestinal brush-border sugar and amino acid transport in carnivores. Am. J. Physiol. 261:R793-801.

Buddington, R. K., and J. M. Diamond. 1989. Ontogenetic development of intestinal nutrient transporters. Ann. Rev. Physiol. 51:601-619.

Burnett, G. S. 1966. Studies of viscosity as the probable factor involved in the improvement of certain barely for chicken by enzyme supplementation. Brit. Poultr. Sci. 7:55-75.

Carruthers, A. 1990. Facilitated diffusion of glucose. Physiol. Rev. 70:1135-1176.

Casewell, L. F. 1990. Fungal additives. Issue of Feed Management.

Caton, J. S., D. O. Erickson, D. A. Carey, and D. L. Ulmer. 1993. Influence of *Aspergillus* fermentation extract on forage intake, site of digestion, in situ degradability, and duodenal amino acid flow in steers grazing cool-season pasture. J. Anim. Sci. 71:779-787.

Chah, C. C., C. W. Carlson, G. Semeniuk, I. S Palmer, and C. W. Hesseltine. 1975. Grow-promoting effect of fermented soybeans for broilers. Poultr. Sci. 54:600-609.

Chen, C. C., T. Tsuchiya, Y. Tamnae, J. M. Wood, and T. H. Wilson. 1985. Na^+ (Li^+)-proline cotransport in *Escherichia coli*. J. Memb. Biol. Soc. Lett. 148:35-42.

Cheeseman, C. I. 1986. Expression of amino acid and peptide transport systems in rat small intestine. Am. J. Physiol. 251:G636-641.

Cheesemen, C. I., and B. Harley. 1991. Adaptation of glucose transport across rat enterocytes basolateral membrane in response to altered dietary carbohydrate intake. J. Physiol (Lond.). 437:563-575.

Christensen, H. N. 1984. Organic ion transport during the seven decades: The amino acids. Biochim. Biophys. Acta 779:255-269.

Christiansen, M. L., and K. E. Webb, Jr. 1990a. Nitrogen utilization and digestibility of amino acid by lambs fed a high-concentrate diet with limestone or magnesium oxide. J. Anim. Sci. 68:2095-2104.

Christiansen, M. L., and K. E. Webb, Jr. 1990b. Intestinal acid flow, dry matter, starch and protein digestibility and amino acid absorption in beef cattle fed a high-concentrate diet with deflorinated phosphate, limestone or magnesium oxide. J. Anim. Sci. 68:2105-2118.

Cohen, L. L., and K. C. Huang. 1964. Intestinal transport of tryptophan and its derivatives. Am. J. Physiol. 206:647-652.

Collington, G. K., D. S. Parker, and D. G. Armstrong. 1988. The influence of probiotics or Tylosin on growth of pigs and development of the gastrointestinal tract. Anim. Prod. 46:521-522.

Collington, G. K., D. S. Parker, and D. G. Armstrong. 1990. The influence of inclusion of either an antibiotic or probiotic in the diet on the development of digestive enzyme activity in the pig. Br. J. Nutr. 64:59-70.

Corcelli, A., and C. Storelli. 1983. The role of potassium and chloride ions on the Na^+/acidic amino acid cotransport system in rat intestinal brush border membrane vesicles. Biochim. Biophys. Acta. 732:24-31.

Crane, R. K., D. Miller and I. Bihler. 1961. The restrictions on possible mechanisms of active transport of sugars. In: A. Kleinzeller, and A. Kotyk (Ed.) Membrane transport and Metabolism. pp 439-449. Prague, Czech. Acad. Sci.

Curran, P. T., S. G. Schultz, R. A. Chez and R. E. Fuiazi. 1967. Kinetics relations of the Na^+-amino acid interaction at the mucosal border of interaction. J. Gen. Physiol. 50:1261-1286.

Dahlqvist, A. 1968. Assay of the intestinal disaccharidase. Anal. Biochem. 22:99-107.

Daniels, L. B., and R. B. Hashim. 1977. Evaluation of fungal cellulase in rice hull base diets for ruminants. J. Dairy Sci. 60:1563-1567.

Darnell, J. E., H. F. Lodish, and D. Baltimore, 1990. Transport across cell membrane. In: J. E. Darnell, H. F. Lodish, and D. Baltimore, (Ed.) Molecular cell biology pp 531-581. Scientific American books, New York.

Denigan, M. E., J. T. Huber, G. Alhadhrami, and A. Al-Dehneh. 1992. Influence of feeding varying levels of Amaferm on performance of lactating dairy cows. J. Dairy Sci. 75:1616-1621.

Dhaunsi, G. S., G. S. Sidhu, U. C. Garg, Y. Singh, R. N. Chakravarti, and R.

Bhatnagar, 1989. Effects on intestinal nutrient uptake and brush border membrane enzymes in response to atherogenic diets in rhesus monkeys. J. Med. Primatol. 18:467-475.

Diamond, J. M., and W. H. Karasov, 1984. Effect of dietary carbohydrate on monosaccharide uptake by mouse small intestine in vitro. J. Physiol. 349:419-440.

Dierick, N. A., I. J. Vervaeke, J. A. Decuypere, and H. K. Henderickx. 1986. Influence of the gut flora and of some growth-promoting feed additives on nitrogen metabolism in pigs. I. Studies in vitro. Livestock Prod. Sci. 14:161-176.

DiRienzo, D. B. 1990. Free and peptide amino acid fluxes across the mesenteric and non-mesenteric viscera of sheep and calves, Ph.D. Diss., Virginia Polytechnic Institute and State University, Blacksburg, 1990.

Droniuk, R., P. T. S. Wong, G. Wisse, and R. A. MacLeod. 1987. Variation in quantitative requirement for Na^+ for transport of metabolizable compounds for by the marine bacteria *Alteromonas halopanktis* 214 and *vibrio fischeri*. Appli. Environ. Microbiol. 53:1487-1495.

Ebil, H., and W. E. Lands. 1969. A new, sensitive determination of phosphatase. Anal. Biochem. 30:51-57.

Ecknauer, R., T. Vadakel, and R. Wepler. 1982. Intestinal morphology and cell production rate in aging rats. J. Gerontol. 37:151-155.

Erni, B. 1989. Glucose transport in *Escherichia coli*. FEMS Microbiol. Rev. 63:13-24.

Fahey, G. C., L. L. Gerger. 1988. Carbohydrate nutrition of ruminants. In: D. C. Church. The Ruminant Animal: Digestive Physiology and Nutrition. pp 269-297.A Reston Book, Prentice Hall, Englewood Cliffs NJ.

Fedorak, R. N. 1990. Adaptation of small intestinal membrane transport process during diabetes mellitus in rats. Can. J. Physiol. Pharmacol. 68:630-635.

Ferraris, R. P., and J. M. Diamond. 1989. Specific regulation of intestinal nutrient transporters by their dietary substrates. Annu. Rev. Physiol. 51:125-141.

Ferraris, R. P., and J. M. Diamond. 1992. Crypt-villus site of glucose transporter induction by dietary carbohydrate in mouse intestine. Am. J. Physiol. 262:G1069-1073.

Ferraris, R. P., S. A. Villenas, and J. M. Diamond. 1992a. Regulation of brush-border enzymes activities and enterocytes migration rated in mouse small intestine. Am. J. Physiol. 262:G1047-1059.

Ferraris, R. P., S. A. Villenas, B. A. Hirayama, and J. M. Diamond. 1992b. Effect of diet on glucose transporter site density along the intestinal crpt-villus

axis. Am. J. Physiol. 262:G1060-1068.

Ferraris, R. P., S. Yasharpour, K. C. K. Lloyd, R. Mirzayan, and J. M. Diamond. 1990. Luminal glucose concentrations in the gut under normal conditions. Am. J. Physiol. 259:G822-837.

Firkins, J. L., W. P. Weiss, M. L. Eastridge, and B. L. Hull. 1990. Effects of feeding fungal culture extract and animal-vegetable fat on degradation of hemicellulose and on ruminal bacterial growth in heifers. J Dairy Sci. 73:1812-1822.

Fondevila, M.. C. J. Newbold, P. M. Hotten, and E. R. Orskov. 1990. A note on the effect of *Aspergillus oryzae* fermentation extract on the rumen fermentation of sheep fed straw. Anim. Prod. 51:422-425.

Fredlander, M., M. Muecker. 1992. International Cytology. A Survey of Cell biology. Vol. 137A. pp 1-35. Academic Press, Inc. New York.

French, D. 1973. Chemical and physical properties of starch. J. Anim. Sci. 37:1048-1061.

Frumholtz, P. P., C. J. Newbold, and R. J. Wallace. 1989. Influence of *Aspergillus oryzae* fermentation extract of a basal ration in the rumen simulation technique (Rusitec). J. Agric. Sci. 113:169-172.

Fujiata, M., H. Ohta, K. Kawai, H. Matsui, and M. Nakao. 1972. Differential isolation of microvillus and basolateral plasma membranes from intestinal mucosa: Mutually exclusive distribution of digestive enzyme and ouabain-sensitive ATPase. Biochim. Biophys. Acta 274:336-347.

Ganapathy, V., and F. H. Leibach. 1985. Is intestinal peptide transport energized by a proton gradient? Am. J. Physiol. 249: G153-160.

Garnner, D. K. 1990. Biochemistry of extracelluar and intercellular communication. In: R. K. Murray, P. A. Mayes, D. K. Granner and V. W. Rodwell (Ed.) Harper's Biochemistry 22nd. pp 441-466. Appleton and Lange, Norwalk, Connecticut/ san Mateo, California.

Goh, Y. K., A.. Shires, A. R. Robblee, and D. R. Clandinn. 1982. Influence of supplementary cellulolytic enzymes on the performance of broiler feed rations containing rapeseed meal. Can. J. Anim. Sci. 62:269-273.

Gomez-Alarcon, R. A., C. Dudas, and J. T. Huber. 1990. Influence of culture of *Aspergillus oryzae* on rumen and total tract digestibility of dietary components. J. Dairy Sci. 73:703-710.

Gomez-Alarcon, R. A., J. T. Huber, G. E. Higginbotham, F. Wiersma, D. Ammon, and B. Taylor. 1991. Influence of feeding *Aspergillus oryzae* fermentation extract on the milk yields eating pattern, and body temperatures of lactating cows. J. Dairy Sci. 69:1733-1740.

Gould, G. W., and G. I. Bell. 1990. Facilitative glucose transporters: an expanding family. Trends Biochem. Sci. 15:18-23.

Gray, G. M. Carbohydrate absorption and malabsorption. 1981. In: L. R. Johnson (Ed.) Physiology of the Gastrointestinal Tract, pp 1063-1072. Raven Press, New York.

Guerino, F., and C. R. Baumrucker. 1987. Identification of methionine and lysine transport system in cattle small intestine. J. Anim. Sci. 65:630-640.

Hampson, D. J., and W. C. Smith. 1986. Influence of creep feeding and dietary intake after weaning on malabsorption and occurrence of diarrhea in the newly weaned pig. Res. Vet. Sci. 41:63-69.

Haresign, W. and W. Ewing. 1989. Review of probiotics products available in the UK. In: Probiotics (Theory and Application). Chalcombe Publications.

Harig, J. M., J. A. Barry, V. M. Rajendran, K. H. Soergel, and K. Ramaswamy. 1989. D-Glucose and L-leucine transport by human intestinal brush-border membrane vesicles. Am. J. Physiol. 256:G618-623.

Hatfield, E. E., and D. L. Hixon. 1975. Dietary additive for new feeder cattle. Illinois Beef Day, Illinois Beef Day AS-669h. University of Illinois , Champaign-Urbana.

Hediger, M. A., M. Coady, T. Ikeda, and E. M. Wright. 1987. Expression cloning and cDNA sequencing of the Na^+/glucose cotransporter. Nature (Lond.) 330:379-381.

Hediger, M. A., E. Turk, and E. Wright. 1989. Homology of the human intestinal Na^+/glucose and E. coli Na^+/proline cotransporters. Proc. Natl. Acad. Sci. USA. 86:5748-5752.

Henderson, P. T. F., S. A. Balwin, and M. T. Cairns, 1992. Sugar-cation symport systems in bacteria. In: M. Friedlander and M. Mueckler (Ed.) International Review of Cytology. A Survey of Cell biology. vol 137A pp 149-208. Academic Press. Inc. New York.

Herring, M. A., and D. M. Hallford. 1991. Growth and carcass characteristics and serum growth hormone, insulin and prolactin in lambs supplemented with amaferm. SID Sheep Res. J. 6(2):5-9.

Higginbotham, G. E., D. L. Bath, and L. J. Butler. 1993. Effect of Feeding an *Aspergillus oryzae* Extract on milk production and related responses in a commercial dairy herd. J. Dairy Sci. 76:1484-1489.

Hopfer, M. 1981. Transport across biological membranes. Pitman Publ. Ltd., Bath, UK.

Hopfer, U. 1987. Membrane transport mechanisms for hexoses and amino acids in the small intestine. In: L. R. Johnson (Ed.) Physiology of the

Gastrointestinal Tract, Vol. II, pp 1499-1526. Raven Press, New York.

Jahn, E., T. P. Chandler, and C. N. Miller. 1973. Lactational responses of cows inoculated with live adapted rumen microorganisms. J. Dairy Sci. 56:643.

James, P. S., M. W. Smith., D. R. Tivey, and T. J. G. Wilson. 1987. Epidermal growth factor selectively increases maltase and sucrase activities in neonatal piglet intestine. J. Physiol. 393:583-594.

Janes, A. N., T. E. C. Weekes, and D. G. Armstrong. 1985. Absorption and metabolism of glucose by mesenteric-drained viscera of sheep fed a dried-grass or ground maize-based diets. Br. J. Nutr. 54:449-458.

Johns, J. T., and W. G. Bergen. 1973. Studies on amino acid uptake by ovine small intestine. J. Nutr. 103:1581-1586.

Jorgensen, P. L. 1975. Techniques for the assay of steroid effect on membranous Na^+/K^+ATPase. In: B. W. O'Malley and J. G. Hardman (Ed.) Methods in Enzymology . Vol. 36(36) pp 434-439. Academic Press, New York.

Judkins, M. B., and R. H. Stobart. 1988. Influence of two levels of enzyme preparation on ruminal fermentation, particulate and fluid passage and cell wall digestion in weather lambs consuming either a 10 % or 24 % grain diet. J. Anim. Sci. 66:1010-1015.

Karasov, W., H, R. S. Pond III, D. H. Solberg, and J. M. Diamond. 1983. Regulation of proline and glucose transport in mouse intestine by dietary substrate levels. Proc. Natl. Acad. Sci. USA. 80:7674-7677.

Karasov, W., H, D. H. Solberg, S. Carter, M. Hughes, D. Phan, F. Zollman, and J. M. Diamond. 1986. Uptake pathways for amino acid in mouse intestine. Am. J. Physiol. 251:G501-508.

Kaunitz, J. D., and E. M. Wright. 1983. Heterogeneity of the intestinal sodium D-glucose carrier. Fed. Proc. 42 (5): 1287 (Abstr.).

Kaunitz, J. D., and E. M. Wright. 1984. Kinetics of sodium D-glucose cotransport in bovine intestinal brush border vesicles. J. Membrane Biol. 79:41-51.

Keelan, M., A. B. R. Thomson., M. L. Grag, and M. T. Clandinin. 1990. Critical period programming of intestinal glucose transport via alterations in dietary fatty acid composition. Can. J. Physiol. Pharmacol. 68:642-645.

Keljo, D. J., R. J. MacLeod, M. H. Perdue, D. G. Butler, and J. R. Hamilton. 1985. D-glucose transport in piglet jejunal brush border membranes: insight from a disease model. Am. J. Physiol. 249:G751-760.

Kellems, R. O., A. Lagerstedt, and M. V. Wallentine. 1990. Effect of feeding *Aspergillus oryzae* fermentation extract or *Aspergillus oryzae* plus yeast culture plus mineral and vitamin supplement on performance of holstein cows during a complete lactation. 1990. J. Dairy Sci. 73:2922-2928.

Kessler, M., O. Acuto, C. Storrelli, H. M. Murer, and G. Semenza. 1978. A modified procedure for the rapid preparation of efficiently transporting vesicles from small intestinal brush border membranes: their use in investigating some properties of D-glucose and cholic transport systems. Biochim. Biophys. Acta 506:136-155.

Kessler, M., and G. Toggenburger. 1979. Non-electrolyte transport in small intestinal membrane vesicles. The application of filtration for transport and binding studies. In: E. Carafoli and G. Semenza (Ed.) Membrane Biochemistry: A Laboratory Manual for Transport Bioenergetics. pp 1-24. Springer-Verlag, New York.

Kimmich, G. A. 1981. Intestinal absorption of sugar. In: L. R. Johnson, (Ed.) Physiology of the Gastrointestinal Tract. Vol. 2. pp 1035-1061, Raven Press, New York.

Koeln et al, L. L., T. S. Schlaghech, and K. E. Webb, Jr. 1993. Amino acid flux across the gastrointestinal tract and liver of calves. J. Dairy Sci. 76(8):2275-2285.

Kreikemeier, K. K., D. L. Harmon, R. T. Brandt, Jr., T. B. Avery, and D. E. Johnson. 1991. Small intestinal starch digestion in steers: Effect of various levels of abomasal glucose, corn starch corn dextrin infusion on small intestinal disappearance and net glucose absorption. J. Anim. Sci. 69:328-338.

Kretchmer, N., J. S. Latimer, F. Raul, K. Berry, C. Legum, and H. L. Sharp. 1979. Sucrase and cellular development. In: Ciba Foundation Symposium. (Ed.) Development of Mammalian Absorptive Processes. pp 117-131. Excerpta Medica, Amsterdam. Oxford. New York.

Kung, Jr., L. K., A. O. Hession, R. S. Tung, and E. M. Kreck, 1992. Directed-fed microbials for ruminants. Presented at the California Animal Nutrition Conference. Pioneer Hi-Bred International Inc., Microbial Genetics Division.

Kwan, W. C., G. A. Quamme, and H. J. Freeman. 1987. Sodium D-dependent glucose transport in brush-border membrane vesicles after massive distal small bowel resection in the rat. Gastroenterology 92:1987-1993.

Lash, L. H. and D. P. Jones, 1984. Characteristics of cysteine uptake in intestinal basolateral membrane vesicles. Am. J. Physiol. 247:G394-401.

Macgregor, R. C., and D. A. Armstrong. 1984. The feed antibiotic avoparcin and net uptake of amino acid from the small intestine of sheep. Can. J. Anim. Sci. 64:(Suppl.) 134.

Maenz, D. D., C. Chenu, S. Breton, and A. Berteloot. 1992. pH-dependent heterogeneity of acidic amino acid transport in rabbit jejunal brush border membrane vesicles. J. Biol. Chem. 267:1510-1516.

Malo, C. 1988. Kinetic evidence for heterogeneity in Na^+ -D-Glucose cotransport systems in the normal human fetal small intestine. Biochim. Biophys. Acta. 938:181-188.

Malo, C. 1991. Multiple pathways for amino acid transport in brush border membrane vesicles isolated from human fetal small intestine. Gastroentrology 100:1644-1652.

Malo, C., and A. Berteloot. 1991. Analysis of Kinetics data in transport studies: New insights from kinetic studies of Na^+-D-glucose cotransport in human intestinal brush-border membrane vesicles using a fast sampling, rapid filtration apparatus. J. Membrane Biol. 122:127-141.

Martin, S. A., and D. J. Nisbet. 1990. Effects of *Aspergillus oryzae* fermentation extract on fermentation of amino acids, bermudagrass and starch by mixed ruminal microorganisms in vitro. J. Anim. Sci. 68:2142-2149.

Martin, S. A., and J. B. Russell. 1986. Phosphoenolpyruvate-dependent phospholylation of hexoses by ruminal bacteria: evidence for the phosphotransferase transport system. Appl. Environ. Microbiol. 52:1348-1352.

Master, B. S. S., C. H. Williams, Jr., and H. Kamin. 1967. The preparation and properties of microsomal TPNH-cytochrome c reductase from pig liver. In: R. W. Estabrook and M. E. Pullman (Ed.) Methods in Enzymology. Vol. 10(92). pp 565-573. Academic Press, New York.

Matthews, D. M. 1975. Absorption of peptide by mammalian intestine. In: D. M. Matthews and J. W. Payne (Ed.) Peptide Transport in Protein Nutrition. pp 61-146. American Elsevier, New York.

Matthews, D. M., and L. Laster. 1965. Competition for intestinal transport among five neutral amino acids. Am. J. Physiol. 208:601-606.

Matthews, D.M. and J. W. Payne. 1975. Peptide in the nutrition of microorganisms In: D. M. Matthews and J. W. Payne (Ed.) Peptide Transport in Protein Nutrition. pp 1-55. American Elsevier, New York.

McCarthy, D. M., J. A. Nicholson, and Y. S. Kim. 1980. Intestinal enzyme adaptation to normal diets of different composition. Am. J. Physiol. 239:G445-451.

Meddings, J. B., D. DeSouza, M. Goel, and S. Thiesen. 1990. Glucose transport and microvillus membrane physical properties along the crpt-villus axis of the rabbit. J. Clin. Invest. 85:1099-1107.

Miller, B. G., P. S. James, M. W. Smith, and F. J. Bource. 1986. Effect of weaning on the capacity of pig intestinal villi to digest and absorb nutrients. J. Agric. Sci. (Camb.) 107:579-589.

Moe, A. J., P. A. Pocius, and C. E. Polan. 1985. Isolation and characterization of brush border membrane vesicles from bovine small intestine. J. Nutr. 115:1173-1179.

Moe, A. J., P. A. Pocius, and C. E. Polan. 1987. Transport of L-amino acids by brush border membrane vesicles from bovine small intestine. J. Dairy Sci. 71:290-297.

Moog, F., 1977. The small intestine in old mice: Growth, alkaline phosphatase and disaccharidase activities, and deposition of amyloid. Exp. Geront. 12:223-235.

Moog, F., 1979. The differentiation and redifferentiation of the intestinal epithelium and its brush border membrane. In: Ciba Foundation Symposium. (Ed.) Development of Mammalian Absorptive Processes. pp 31-50. Excerpta Medica. Armsterdam. Oxford. New York.

Moriyama, I. S. 1986. Development of fetal organs and adaptation to extrauterine life. Acta Obstet. Gynecol. Jpn. Engl. Ed. 38:1227-1237.

Moss, B. R., A.. F. Beeckler, C. W. Newman, A. M. El-Negoumy. 1977. Enzyme supplementation of broiler rations. Poutr. Sci. 56:1749.

Mountfort, D. O. 1987. The rumen anaerobic fungi. FEMS Microbiol. Rev. 46:401-408.

Munck, B. G. 1980. Lysine transport across the small intestine. Stimulating and inhibitory effects of neutral amino acids. J. Membr. Biol. 53:45-53.

Munck, B. G. 1981. Intestinal absorption of amino acids. In: L. R. Johnson, S. G. Schultz. (Ed.) Physiology of the gastrointestinal tract vol II. pp 1097-1122, Raven Press, New York.

Munck, B. G. 1983. Comparative aspects of amino acid transport in guinea pig, rabbit, and rat small intestine. In: M. Gilles-Baillien and R. Gilles, (Ed.) Intestinal Transport. pp 260-283 Springer-Verae, Berlin.

Munck, B. G. 1985. Transport of neutral and cationic amino acids across the brush border membrane of the rabbit ileum. J. Membr. Biol. 83:1 -13.

Munck, L. K, and B. G. Munck. 1992. Variation in amino acid transport along the rabbit small intestine. Mutual jejunal carriers of leucine and lysine. Biochim. Biophys. Acta. 1116:83-90.

Munck, L. K, and B. G. Munck. 1992. The rabbit 'imino carrier' and the ileal 'imino acid carrier' describe the same epithelial function. Biochim. Biophys. Acta. 1116:91-96.

Munck, B. G., and S. N. Raamussen. 1979. Lysine transport across rat jejunum transport . J. Physiol. (Lond.) 291:291-303.

Newbold, C. J., R. Brock, and R. J. Wallace. 1991. Influence of autoclaved or irradiated *Aspergillus oryzae* fermentation extract on fermentation in the rumen simulation technique (Rusitec). J. Agri. Sci. (Lond.). 116:159-162.

Nisbet, D. J., and S. A. Martin. 1990. Effect of dicarboxyric acids and *Aspergillus oryzae* fermentation extract on lactate uptake by the ruminal bacterium *Selenomonas ruminantium*. Appl. Environ. Microbiol. 56:3515-3518.

NRC. 1988. Nutrient Requirements of Swine (9th Ed.) National Academy Press, Washington, DC.

Oellermann, S. O., M. J. Arambel, B. A. Kent, and J. L. Walters. 1990. Effect of graded amount of *Aspergillus oryzae* fermentation extract on ruminal characteristics and nutrient digestibility in cattle. J. Dairy Sci. 73:2413-2416.

Ogura, M., H. Ogura, S. Ikehara, M. L. Dao, and R. A. Good. 1989. Decrease by chronic energy intake restriction of celluar proliferation in the intestinal epithelium and lymphoid organs in autoimmunity-prone mice. Proc. Natl. Acad. Sci. USA. 86:5918-5922.

Ohkohchi, N., T. Andoh, R. Ohi, and S. Mori. 1990. Defined formula diets alter characteristics of the intestinal transport of amino acid and peptides in growing rats. J. Pediatric Gastroenterology Nutr. 10:490-496.

Owens, F. N., R. A. Zinn, and Y. K. Kim. 1986. Limits to starch digestion in the ruminant small intestine. J. Anim. Sci. 63:1634-1648.

Oxender, D. L., Lee, P. A. Moore, and G. Cecchini. 1977. Neutral amino acid transport systems of tissue culture cells. J. Biol. Chem. 252:2675-2679.

Parker, D. S. 1990. Manipulation of the functional activity of the gut by dietary and other means (antibiotics/probiotics) in ruminants. J. Nutr. 120:639-648.

Parker, D. S., and D. G. Armstrong. 1987. Antibiotic feed additives and livestock production. Proc. Nutr. Soc. 46:415-421.

Paterson, J. Y. F., F. V. Sepulveda, and M. W. Smith. 1981. Distinguishing transport systems having overlapping specificities for neutral and basic amino acids in the rabbit ileum. J. Physiol. (Lond.) 319:345-354.

Phillips, W. A., K. E. Webb, Jr., and J. P. Fontenot. 1976. In vitro absorption of amino acids by the small intestine of sheep. J. Anim. Sci. 42:201-207.

Phillips, W. A., K. E. Webb, Jr., and J. P. Fontenot. 1979. Characteristic of threonine, valine and methionine absorption in the jejunum and ileum of sheep. J. Anim. Sci. 48:926-933.

Prange, R. W., M. D. Stern, L. M. Rode, K. A. S. Santos, N. A. Jorgensen, and L. D. Satter. 1979. The effects of altering hay: grain ratios on digestibility and rate of passage of dry matter in lactating dairy cattle. J. Anim. Sci. 49(1):39

(Abstr.).

Reese, G. L., R. S. Karg, B. I. Fancher, and P. W. Waldroup. 1983. The use of supplemental enzymes in the diets of broiler chicks. Nutr. Report Int. 28:919-929.

Rexen, B. 1981. Use of enzymes for improvement of feed. Anim. Feed Sci. Technology. 6:105-114.

Russell, J. B., H. J. Strobel, and S. A. Martin. 1990. Strategies of nutrient transport by ruminal bacteria. J. Dairy Sci. 73:2996-3012.

Russell, J. R., A. W. Young, and N. A. Jorgensen. 1981. Effect of dietary corn starch intake on pancreatic amylase and intestinal maltase and pH in cattle. J. Anim. Sci. 52:1177-1182.

SAS. 1988. SAS Use's Guide: Statistics Release 6.03 (Ed.). SAS Inc., cary, NC.

Satoh, O., S. Koyama, K. Yamada, and T. Kawasaki. 1991. Changes in amino acid glucose transport in brush border membrane vesicles of hyperglycemic guinea-pig small intestine. Biochim. Biophys. Acta. 1063:155-161.

Scharrer, E., W. Peter, and W. Raad. 1979. Reciprocal relationship between rumen development and intestinal sugar transport capacity in sheep. Zbl. Vet. Med. A26:513-520.

Schedl, H. P., C. E. Pierce, A. Rider, and J. A. Clifton. 1968. Absorption of L-methionine from human small intestine. J. Clin. Invest. 47:417-425.

Schultz, S. G., and P. F. Curran. 1970. Coupled transport of sodium and organic solutes. Physiol. Rev. 50:637-718.

Seal, C. J., and D. S. Parker, 1992. Isolation and characterization of circulating low molecular weight peptides in steer, sheep, and rat portal and periperal blood. Comp. Biochem. Physiol. 99B:679-685.

Sepulveda, F. V. and M. W. Smith. 1979. Different mechanisms for neutral amino acid uptake by new-born pig colon. J. Physiol. 286:479-490.

Semenza, G., M. Kessler, M. Hosang, and U. Schmidt. 1984. Biochemistry of the Na^+, D-glucose cotransporter of the small intestinal brush-border membrane. Biochim. Biophys. Acta. 779:343-379.

Shirazi-Beechey, S. P., B. A. Hirayama, Y. Wang, D. Scott, M. W. Smith, and E. M. Wright. 1991a. Ontogenetic development of lamb intestinal sodium-glucose co-transport is regulated by diet. J. Physiol. 437:699-708.

Shirazi-Beechey, S. P., M. W. Smith, Y. Wang, and P. S. James. 1991b. Postnatal development of lamb intestinal digestive enzymes is not regulated by diet. J. Physiol. (Lond.). 437:691-698.

Silk, D. B. A., P. D. Fairclough, M. L. Clark, J. E. Hegarty, T. C. Marrs, J. M. Addison, D. Burston, K. M. Clegg, and D. M. Matthews. 1980. Use of a peptide rather than free amino acid nitrogen source in chemically defined elemental diets. J. parent. Ent. Nutr. 4:548-553.

Slippers, S., E. L. Steenkamp, and J. F. Naude. 1988. The effect of various Bospro inclusion rates in feedlot rations with different roughage levels on the performance of steers. Proc. 27th Congress of the South African Society of Animal Production.

Spears, J. W. 1990. Ionophores and nutrient digestion and absorption in ruminants. J. Nutr. 120:632-638.

Spencer, R., and A. H. Samiy. 1961. Intestinal absorption of L-phenylalanine in vitro. Am. J. Physiol. 200:501-504.

Stein, E. D., S. D. Chang, and J. M. Diamond. 1987. Comparison of different dietary amino acids as inducers of intestinal amino acid transport. Am. J. Physiol. 252:G626-635.

Stevens, B. R., J. D. Kaunitz, and E. M. Wright. 1984. Intestinal transport of amino acids and sugars: Advances using membrane vesicles. Ann. Rev. Physiol. 46:417-433.

Stevens, B. R., H. J. Ross, and E. M. Wright. 1982. Multiple transport pathways for neutral amino acids in rabbit jejunal brush border vesicles. J. Membr. Biol. 66:213-225.

Stingelin, Y., E. Scharrer and S. Wolffram. 1986. Duodenal absorption of amino acids in sheep and its dependence on sodium and pH. J. Anim. Physiol. Nutr. 56:67-72

Storrie, B., and E. A. Madden. 1990. Isolation of subcelluar organelles. In: M. P. Deutscher (Ed.) Methods of Enzymology. Vol. 182(16). pp 203-225. Academic Press, New York.

Strobel, H. J., and J. B. Russell. 1991. Role of sodium in the growth of a ruminal *Selenomoad.* Appl. and Enviro. Microbiol. 57:1663-1668.

Suga, Y., M. Kawai., S. Noguchi., G. Shimura and H. Samejima. 1978. Application of cellulolytic and plant tissue macerating enzyme of Irpex lacteus Fr. as feed additive enzyme. Agri. Biol. Chem. 42:347-350.

Tegbe, S., and Zimmerman, D. R. 1977. Evaluation of a yeast single cell protein in pig diets. J. Anim. Sci. 45:1309-1316.

Thomson, A. B. R., M. Keelan, M. T. Clandinin, and K. Walker. 1987. A high linoleic acid diet diminishes enhanced intestinal uptake of sugars in diabetic rats. Am. J. Physiol. 252:G262-271.

Thomson, A. B. R., M. Keelan, M. Garg, and M. T. Clandinini. 1989. Evidence of

critical programming of intestinal transport function: variations in the dietary ratio of polyunsaturated fatty acids alter ontogeny of the rat intestine. Biochim. Biophys. Acta 1001:302-315.

Titus, E., W. H. Karasov, and G. A. Ahearn. 1991. Dietary modulation of intestinal nutrient transport in the teleost fish tilapia. Am. J. Physiol. 261:R1568-1574.

Tuba, J., and N. Dickie. 1955. The role of intestinal alkaline phosphatase in intestinal absorption. IV. The effect of various proteins on levels of the enzyme in intestinal mucosa. Can. J. Biochem. Physiol. 33:89-92.

Ustyuzhanina, S. V., V. L. Yarovenko, and I. N. Voinarskii. 1984. Synthesis of protease and alpha-amylase by the fungus. Prikladnaya Biokhimiya i Mikrobiologiya. 20(5):616-621.

Van Horn, H. H., B. Harris, Jr., M. J. Taylor, K. C. Bachman, and C. J. Wilcox. 1984. By-product feeds for lactating dairy cows: effects of cottonseed hulls, sunflower hulls, corrugated paper, peanut hulls, sugarcane bagasse, and whole cotton seed with additives of fat, sodium bicarbonate, and *Aspergillus oryzae* product on milk production. J. Dairy Sci. 67:2922-2938.

Vega, Y. M., A. A. Puchal, and R. K. Buddington. 1992. Intestinal amino acid and monosaccharide transport in suckling pigs fed milk replaces with different sources of carbohydrate. J. Nutr. 122:2430-2439.

Webb, Jr., K. E. 1986. Amino acid and peptide absorption from the gastrointestinal tract. Fed. Proc. 45:2268-2271.

Webb, Jr., K. E. 1990. Intestinal absorption of protein hydrolysis products: A review. J. Anim. Sci. 68:3011-3022.

Webb, Jr., K. E. 1992. The potential impact of proteolysis in high-protein silages on peptide absorption. Presented at the California Animal Nutrition Conference. Pioneer Hi-Bred International Inc. Microbial Genetics Division.

Weekes, T. E. C. 1979. Carbohydrate metabolism. In: D. C. Church (Ed.) Digestive Physiology and Nutrition of Ruminants. pp 187-209. O & B Books, Corvallis, OR.

White, W. B., H. R. Bird, M. L. Sunde, N. Prentice, W. C. Burger, and J. A. Marlett. 1981. The viscosity interaction of barley beta-glucan with *trichoderma virde* cellulase in the chick intestine. Poultr. Sci. 60:1043-1048.

Wiedmeier, R. D., M. J. Arambel, and J. L. Walters. 1987. Effect of yeast culture and *Aspergillius oryzae* fermentation extract on ruminal characteristics and nutrient digestibility. J. Dairy Sci. 70:2063-2068.

Williams, P. E. V., C. A. G. Tait, G. M. Inners, and C. J. Newbold. 1990. Effect of

the inclusion of yeast culture (*Saccharomyces Cerevisiae* plus growth medium) in the diet of dairy cows on milk yield and forage degradation and fermentation patterns in the rumen of steers. J. Anim. Sci. 69:3016-3026.

Wilson, J. W., and K. E. Webb, Jr. 1990a. Lysine and methionine transport by bovine jejunal and ileal brush border membrane vesicles. 68:504-514.

Wilson, J. W., and K. E. Webb, Jr. 1990b. Simultaneous isolation and characterization of brush border membrane vesicles from bovine small intestine. J. Anim. Sci. 68:583-590.

Wolffram, S., E. Eggenberger, and E. Scharrer. 1986. Kinetics of D-glucose and L-leucine transport into sheep and pig intestinal brush border membrane vesicles. Comp. Biochem. Physiol. 84A:589-593.

Wolffram, S., E. Eggenberger, and E. Scharrer. 1989. Kinetics of D-glucose transport across the intestinal brush-border membrane of the cat. Comp. Biochem. Physiol. 94A:111-115.

Wright, E. M., and B. E. Peerce, 1984. Identification and conformational changes of the intestinal proline carrier. Ann. N. Y. Acad. Sci. 456:108-114.

Wright, E. M., E. Turk, B. Zabel, S. Mundlos, and J. Dyer. 1991. Molecular genetics of intestinal glucose transport. J. Clin. Invest. 88:1435-1440.

Wright, E. M., C. H. Van Os, A. K. Mircheff. 1980. Sugar uptake by intestinal basolateral membrane vesicles. Biochim. Biophys. Acta 597:112-124.

Yeh, K. Y., and P. R. Holt, 1986. Ontogenetic timing mechanism initiates the expression of rat intestinal sucrase activity. Gastroenterology 90:520-526.

Zamora, R. G., and T. L. Veum. 1979. The nutritive value of dehulled soybeans fermentated with *Aspergillus oryzae* or *Rhizopus oligosporus* as evaluated by rats. J Nutr. 109:1333-1339.

Zamora, R. G., and T. L. Veum. 1988. Nutritive value of whole soybeans fermentated with *Aspergillus oryzae* or *Rhizopus oligosporus* as evaluated by neonatal pigs. J Nutr. 118:438:444.

Chapter IV

Appendix

Appendix Table 1. Total amount of protein and percent recovery from homogenate for pig intestinal brush border membrane fractions (n=4)

Fraction	Protein	SD	Recovery[a]	SD	Sum[b]
	----- mg -----		--------- % --------		
Homogenate	2,453.0	271.9	100		
Pellet(P1)	1,112.8	132.3	45.63	6.43	
Supernatant(S1)	1,076.2	208.2	43.63	4.66	89.2
P2a	102.6	9.6	4.20	.38	
S2a	901.4	133.8	36.71	3.71	93.3
P2b	64.1	4.8	2.63	.32	
S2b	25.3	3.8	1.03	.15	87.3
P3	33.5	9.7	1.36	.37	
S3	31.5	6.6	1.31	.36	101.4
P4	21.1	2.4	.87	.15	
S4	5.9	1.1	.25	.06	85.7
Band (B0)	.5	.1	.02	.01	
B1	4.6	1.0	.19	.05	
B2	3.3	.7	.14	.04	
B3	9.8	2.2	.40	.09	86.3

[a]Expresses recovery with respect to the original homogenate. (Example: % recovery for P3 (33.5 mg) = total protein of P3 divided by total protein of H (2,453 mg) times 100 gives 1.36%.)

[b]Expresses the total percentage recovery of the previous differentiation step (Example: sum for P4 (21.1 mg) and S4 (5.9 mg) divided by S3 (31.5 mg) times 100 gives 85.7%.)

Appendix.Table 2. Total alkaline phosphatase activity and percent recovery from homogenate for pig intestinal brush border membrane fractions (n=4)

Fraction	Total activity	SD	Recovery[a]	SD	Sum[b]
	-- umole/min --		--------- % ---------		
Homogenate	176.3	45.3	100		
Pellet(P1)	93.7	18.50	53.79	4.60	
Supernatant(S1)	82.0	13.50	47.47	7.28	99.7
P2a	31.7	10.10	17.76	2.67	
S2a	35.7	5.10	20.85	3.31	82.4
P2b	23.2	5.88	13.21	1.84	
S2b	6.9	1.63	3.97	.60	95.0
P3	14.0	6.53	8.47	4.98	
S3	12.5	6.58	6.79	2.39	114.2
P4	9.1	1.35	4.94	.74	
S4	2.0	.52	1.14	.02	88.8
Band (B0)	.1	.02	.05	.02	
B1	4.7	.80	2.70	.32	
B2	4.1	1.04	2.31	.45	
B3	4.6	2.68	2.50	.77	148.8

[a]Expresses recovery with respect to the original homogenate. (Example: % recovery for P3 (14.0 umol/min) = total activity of P3 divided by total activity of H (176.3 umol/min) times 100 gives 8.47%.)

[b]Expresses the total percentage recovery of the previous differentiation step (Example: sum for P4 (9.1 umole/min) and S4 (2.0 umole/min) divided by S3 (12.5 umole/min) times 100 gives 88.8%.)

Appendix Table 3. Total sucrase activity and percent recovery from homogenate for pig intestinal brush border membrane fractions (n=4)

Fraction	Total activity	SD	Recovery[a]	SD	Sum[b]
	--- umol/min ----		--------- % ----------		
Homogenate	15,045.4	2,827.1	100		
Pellet(P1)	9,192.3	1,813.6	62.80	6.71	
Supernatant(S1)	6,363.0	1,562.2	41.96	3.18	103.4
P2a	3,743.3	743.4	24.94	2.73	
S2a	2,082.9	842.6	13.45	3.44	91.6
P2b	3,115.7	323.5	21.12	3.23	
S2b	562.7	100.3	3.77	.51	98.3
P3	1,346.2	437.2	9.08	3.10	
S3	1,466.5	527.8	9.70	3.26	90.3
P4	1,188.9	364.1	8.04	2.59	
S4	110.8	29.6	.73	.11	88.6
Band(B0)	5.4	3.9	.04	.03	
B1	343.9	80.2	2.32	.51	
B2	412.7	95.0	2.83	.82	
B3	348.0	158.1	2.36	1.11	93.4

[a]Expresses recovery with respect to the original homogenate. (Example: % recovery for P3 (1,346.2 umol/min) = total activity of P3 divided by total activity of H (15,045 4 umol/min) times 100 gives 9.08%.)

[b]Expresses the total percentage recovery of the previous differentiation step (Example: sum for P3 (1,346.2 μmole/min) and S3 (1,466.5 μmole/min) divided by P2b (3,115.7 μmole/min) times 100 gives 90.3%.)

Appendix Table 4. Total acid phosphatase activity and percent recovery from homogenate for pig intestinal BBMV (n=4)

Fraction	Total activity	SD	Recovery[a]	SD	Sum[b]
	------ U ------		--------- % ---------		
Homogenate	23.63	4.55	100		
Pellet(P1)	11.70	1.11	50.72	9.81	
Supernatant(S1)	10.88	1.62	46.38	2.95	95.6
P2a	1.71	.30	7.30	.86	
S2a	8.80	1.61	37.29	1.13	96.6
P2b	1.19	.15	5.13	.79	
S2b	.42	.03	1.83	.38	94.2
P3	.53	.09	2.30	.64	
S3	.66	.03	2.85	.46	100.0
P4	.47	.07	2.02	.20	
S4	.10	.03	.46	.21	86.4
Band(B0)	.01	.001	.05	.01	
B1	.09	.03	.39	.07	
B2	.05	.02	.20	.07	
B3	.23	.03	1.00	.29	80.9

[a]Expresses recovery with respect to the original homogenate. (Example: % recovery for P3 (.53 U) = total activity of P3 divided by total activity of H (23.63 U) times 100 gives 2.30%.)

[b]Expresses the total percentage recovery of the previous differentiation step (Example: sum for P4 (.47 U) and S4 (.10 U) divided by S3 (.66 U) times 100 gives 86.4%.)

Appendix Table 5. Total lactate dehydrogenase activity and percent recovery from homogenate for pig intestinal brush border membrane fractions (n=4)

Fraction	Total activity	SD	Recovery[a]	SD	Sum[b]
	--- ABS U/min ---		--------- % ---------		
Homogenate	15,748	1,755	100		
Pellet(P1)	1,408	215	9.12	2.31	
Supernatant(S1)	14,777	1,152	94.13	3.60	102.8
P2a	279	45	1.76	.11	
S2a	13,804	1,107	88.20	8.93	95.3
P2b	138	27	.87	.12	
S2b	168	29	1.06	.10	109.5
P3	62	2	.40	.06	
S3	75	19	.48	.11	99.8
P4	40	15	.26	.10	
S4	12	3	.08	.01	69.8
Band(B0)	1	0	.01	.03	
B1	15	4	.09	.03	
B2	8	4	.05	.02	
B3	12	5	.08	.03	88.6

[a]Expresses recovery with respect to the original homogenate. (Example: % recovery for P3 (61.8 ABS U/min) = total activity of P3 divided by total activity of H (15,747.5 ABS U/min) times 100 gives .40%.)

[b]Expresses the total percentage recovery of the previous differentiation step (Example: sum for P3 (61.8 ABS U/min) and S3 (75.4 ABS U/min) divided by P2b (137.5 ABS U/min) times 100 gives 99.8 %.)

Appendix Table 6. Total cytochrome c oxidase activity and percent recovery from homogenate for pig intestinal BBMV fractions (n=4)

Fraction	Total activity	SD	Recovery[a]	SD	Sum[b]
	--- ABS U/min ---		--------- % --------		
Homogenate	11,733.6	4,780.1	100		
Pellet(P1)	9,246.7	2,118.7	73.84	20.24	
Supernatant(S1)	503.2	255.5	2.58	.60	83.1
P2a	282.9	47.9	2.58	.60	
S2a	269.6	87.9	2.31	1.79	109.8
P2b	210.4	48.9	1.89	.39	
S2b	43.0	9.6	.4	.15	89.6
P3	109.6	40.5	.98	.36	
S3	87.9	9.2	.81	.22	93.9
P4	51.6	19.6	.45	.15	
S4	6.4	4.4	.05	.03	66.0
Band(B0)	.4	.30	.0006	.002	
B1	1.1	.51	.009	.002	
B2	8.0	3.7	.007	.001	
B3	31.9	10.1	.30	.14	80.3

[a]Expresses recovery with respect to the original homogenate. (Example: % recovery for P3 (109.6 ABS U/min) = total activity of P3 divided by total activity of H (11,733.6 ABS U/min) times 100 gives .98%.)

[b]Expresses the total percentage recovery of the previous differentiation step (Example: sum for P3 (109.6 ABS U/min) and S3 (87.9 ABS U/min) divided by P2b (210.4 ABS U/min) times 100 gives 93.9%.)

Appendix Table 7. Total Na^+/K^+ATPase activity and percent very from homogenate for pig intestinal brush border membrane fractions (n=4)

Fraction	Total activity	SD	Recovery[a]	SD	Sum[b]
	------ U ------		---------- % ---------		
Homogenate	96.45	33.80	100		
Pellet(P1)	62.95	22.32	65.27	23.36	
Supernatant(S1)	38.94	14.03	40.37	27.20	105.6
P2a	8.45	2.54	8.79	2.83	
S2a	16.42	8.42	17.02	3.63	63.9
P2b	7.39	4.41	7.66	4.27	
S2b	.64	.74	.61	.71	95.0
P3	3.14	.75	3.26	3.12	
S3	2.61	.55	2.71	1.00	77.8
P4	1.64	.46	1.70	.71	
S4	.39	.45	.40	.35	64.6
Band(B0)	.00	.00	.00	.00	
B1	.41	.13	.43	.39	
B2	.31	.15	.32	.21	
B3	1.02	.24	.11	.10	106.1

[a]Expresses recovery with respect to the original homogenate. (Example: % recovery for P3 (3.14 U) = total activity of P3 divided by total activity of H (96.45 U) times 100 gives 3.26%.)

[b]Expresses the total percentage recovery of the previous differentiation step (Example: sum for P3 (3.14 U) and S3 (2.61 U) divided by P2b (7.39 U) times 100 gives 77.8%.)

Appendix Table 8. Total cytochrome c reductase activity and percent recovery from homogenate for pig intestinal brush border membrane fractions (n=4)

Fraction	Total activity	SD	Recovery[a]	SD	Sum[b]
	-- ABS U/min --		---------- % ---------		
Homogenate	292.76	65.76	100		
Pellet(P1)	126.67	57.50	41.72	10.69	
Supernatant(S1)	184.12	74.75	61.45	16.30	106.2
P2a	38.41	18.89	12.61	4.14	
S2a	156.71	56.62	52.58	10.92	106.0
P2b	34.60	13.63	11.53	2.61	
S2b	8.34	4.17	2.77	1.10	111.8
P3	16.04	3.88	5.54	1.13	
S3	17.56	7.07	5.89	1.74	97.1
P4	13.77	6.60	2.02	.20	
S4	1.61	.54	.54	.08	87.6
Band(B0)	.14	.04	.05	.02	
B1	1.32	.98	.42	.24	
B2	.97	.74	.31	.18	
B3	7.24	1.18	2.51	.37	70.2

[a]Expresses recovery with respect to the original homogenate. (Example: % recovery for P_3 (16.04 ABS U/min) = total activity of P_3 divided by total activity of H (292.76 ABS U/min) times 100 gives 5.54%.)

[b]Expresses the total percentage recovery of the previous differentiation step (Example: sum for P_3 (16.04 ABS U/min) and S_3 (17.56 ABS U/min) divided by P_{2b} (34.60 ABS U/min) times 100 gives 97.1%.)

Appendix Table 9. Total amount of protein and percent recovery from homogenate for lambs intestinal brush border membrane fractions (n=4)

Fraction	Total protein	SD	Recovery[a]	SD	Sum[b]
	mg		%		
Homogenate	2,727.3	186.5	100		
Pellet(P1)	1,303.5	177.2	47.68	3.97	
Supernatant(S1)	1,168.7	60.4	42.91	1.69	90.6
P2a	139.8	11.1	5.14	.47	
S2a	1,022.4	101.8	37.45	1.77	99.3
P2b	104.2	10.6	3.84	.32	
S2b	28.4	4.1	1.04	.10	94.9
P3	73.5	10.7	2.72	.53	
S3	32.8	4.6	1.20	.17	102.1
P4	26.9	4.0	.99	.18	
S4	10.0	1.2	.37	.03	113.3
Band(B0)	1.0	.3	.04	.01	
B1	5.6	0.8	.21	.03	
B2	4.5	0.8	.17	.03	
B3	10.4	2.0	.38	.09	80.8

[a]Expresses recovery with respect to the original homogenate. (Example: % recovery for P_3 (73.5 mg) = total protein of P_3 divided by total activity of H (2,727.3 mg) times 100 gives 2.72%.)

[b]Expresses the total percentage recovery of the previous differentiation step. (Example: sum for P_4 (26.9 mg) and S_4 (10.0 mg) divided by S_3 (32.8 mg) times 100 gives 113.3%.)

Appendix Table 10. Total Alkaline phosphatase activity and percent recovery from homogenate for lambs intestinal brush border membrane fractions (n=4)

Fraction	Total activity	SD	Recovery[a]	SD	Sum[b]
	--- umole/min --		---------- % ---------		
Homogenate	540.92	628.03	100		
Pellet(P1)	259.53	281.50	49.85	7.39	
Supernatant(S1)	263.35	295.72	50.17	2.59	100.0
P2a	93.34	104.06	17.14	3.27	
S2a	145.98	179.01	28.05	4.40	90.1
P2b	80.17	92.42	15.07	1.20	
S2b	9.59	9.91	2.15	1.40	100.5
P3	52.25	69.42	10.31	2.95	
S3	27.33	26.57	5.99	1.56	108.26
P4	20.79	20.53	4.86	1.21	
S4	4.90	5.23	1.19	0.61	101.0
Band(B0)	.34	.50	.05	.02	
B1	5.76	6.91	1.04	.07	
B2	8.91	1.36	1.36	.39	
B3	7.91	8.28	1.70	.31	85.4

[a]Expresses recovery with respect to the original homogenate. (Example: % recovery for P3 (52.25 umole/min) = total acticity of P3 divided by total activity of H (540.92 umole/min) times 100 gives 10.31%.)

[b]Expresses the total percentage recovery of the previous differentiation step (Example: sum for P4 (20.79 umole/min) and S4 (4.90 umol/min) divided by S3 (27.33 umole/min) times 100 gives 101.0%.)

Appendix Table 11. Total acid phosphatase activity and percent recovery from homogenate for lambs intestinal brush Border membrane fractions (n=4)

Fraction	Total activity	SD	Recovery[a]	SD	Sum[b]
	------ U -------		---------- % ---------		
Homogenate	56.31	18.94	100		
Pellet(P1)	26.76	12.39	46.19	6.41	
Supernatant(S1)	21.04	2.42	41.88	18.19	88.07
P2a	4.87	.45	9.34	3.01	
S2a	14.56	1.60	28.57	11.51	90.52
P2b	3.76	.38	7.16	1.98	
S2b	.90	.16	1.76	.74	95.50
P3	.94	.21	1.89	.89	
S3	2.75	.37	5.26	1.62	99.86
P4	1.88	.21	3.65	1.32	
S4	.33	.08	.65	.27	81.75
Band(B0)	.02	.009	.04	.03	
B1	.24	.02	.46	.14	
B2	.19	.05	.36	.06	
B3	.83	.21	1.26	.47	58.08

[a]Expresses recovery with respect to the original homogenate. (Example: % recovery for P3 (.94 U) = total acticity of P3 divided by total activity of H (56.31 U) times 100 gives 1.89%.)

[b]Expresses the total percentage recovery of the previous differentiation step (Example: sum for P4 (1.88 U) and S4 (.33 U) divided by S3 (2.75 U) times 100 gives 81.75%.)

Appendix Table 12. Total lactate dehydrogenase activity and percent recovery from homogenate for lamb intestinal brush border membrane fractions (n=4)

Fraction	Total activity	SD	Recovery[a]	SD	Sum[b]
	--- ABS U/min ---		---------- % ---------		
Homogenate	9,538.3	417.0	100		
Pellet(P1)	1,407.5	1,090.6	14.45	10.61	
Supernatant(S1)	7,169.9	482.8	74.54	4.40	89.0
P2a	199.3	22.7	2.08	.17	
S2a	6,573.0	834.8	69.09	9.77	95.4
P2b	119.1	12.6	1.25	.01	
S2b	76.6	10.7	.80	.08	98.6
P3	28.0	4.1	.29	.05	
S3	94.4	11.1	.99	.09	102.4
P4	63.4	10.2	.67	.08	
S4	15.7	4.3	.17	.05	84.9
Band(B0)	1.3	.04	.01	.005	
B1	13.3	2.0	.14	.02	
B2	8.8	1.4	.09	.02	
B3	30.3	5.2	.32	.05	83.6

[a]Expresses recovery with respect to the original homogenate. (Example: % recovery for P3 (28.0 ABS U/min) = total acticity of P3 divided by total activity of H (9,538.3 ABS/min) times 100 gives .29%.)

[b]Expresses the total percentage recovery of the previous differentiation step (Example: sum for P3 (28.0 ABS U/min) and S3 (94.4 ABS U/min) divided by P2b (119.1 ABS U/min) times 100 gives 102.4%.)

Appendix Table 13. Total cytochrome c oxidase activity and percent recovery from homogenate for lamb intestinal brush border membrane fractions (n=4)

Fraction	Total activity	SD	Recovery[a]	SD	Sum[b]
	---- ABS U/min ---		--------- % ---------		
Homogenate	12,663.2	6,100.2	100		
Pellet(P1)	9,268.8	3,264.0	76.57	11.44	
Supernatant(S1)	631.0	74.1	5.74	2.17	82.3
P2a	284.3	114.4	2.30	.30	
S2a	303.4	144.3	3.22	2.68	96.2
P2b	202.8	95.3	1.61	.44	
S2b	42.1	22.1	.35	.19	85.2
P3	122.2	64.1	.97	.49	
S3	108.8	51.4	.93	.42	118.0
P4	80.3	29.5	.70	.27	
S4	8.0	3.8	.08	.05	83.9
Band(B0)	.9	.7	.01	.01	
B1	2.9	3.4	.03	.03	
B2	2.1	2.6	.02	.02	
B3	61.6	22.4	.53	.17	84.3

[a]Expresses recovery with respect to the original homogenate. (Example: % recovery for P3 (122.2 ABS U/min) = total acticity of P3 divided by total activity of H (12,663.2 ABS/min) times 100 gives .97%.)

[b]Expresses the total percentage recovery of the previous differentiation step (Example: sum for P3 (122.2 ABS U/min) and S3 (108.8 ABS U/min) divided by P2b (202.8 ABS U/min) times 100 gives 118.0%.)

Appendix Table 14. Total Na^+/K^+ATPase activity and percent recovery from homogenate for lamb intestinal brush border membrane fractions (n=4)

Fraction	Total activity	SD	Recovery[a]	SD	Sum[b]
	------ U -------		--------- % ---------		
Homogenate	76.36	20.17	100		
Pellet(P1)	35.89	13.36	49.85	7.39	
Supernatant(S1)	23.10	4.89	31.22	7.53	81.1
P2a	5.86	2.62	8.00	4.47	
S2a	22.88	3.92	32.56	14.25	129.9
P2b	4.57	3.83	6.50	6.39	
S2b	1.76	1.05	2.29	1.04	109.9
P3	2.65	.49	3.54	.49	
S3	2.34	1.95	3.05	2.06	101.4
P4	.76	.13	1.09	.48	
S4	.58	.68	.74	.85	60.0
Band(B0)	.02	.04	.03	.06	
B1	.23	.10	.31	.14	
B2	.21	.10	.28	.13	
B3	1.01	.30	1.44	.79	189.0

[a]Expresses recovery with respect to the original homogenate. (Example: % recovery for P3 (2.65 U) = total acticity of P3 divided by total activity of H (76.36 U) times 100 gives 3.54%.)

[b]Expresses the total percentage recovery of the previous differentiation step (Example: sum for P3 (2.65 U) and S3 (2.34 U) divided by P2b (4.57 U) times 100 gives 101.38%.)

Appendix Table 15. Total cytochrome c reductase activity and percent recovery from homogenate for lamb intestinal brush border membrane fractions (n=4)

Fraction	Total activity	SD	Recovery[a]	SD	Sum[b]
	--- ABS U/min ---		--------- % --------		
Homogenate	657.53	128.81	100		
Pellet(P1)	299.41	84.54	45.64	11.16	
Supernatant(S1)	287.67	70.59	44.19	10.40	89.8
P2a	70.68	25.01	10.89	3.76	
S2a	202.80	41.07	31.01	4.60	88.8
P2b	52.95	18.64	8.28	3.00	
S2b	17.46	8.90	2.61	1.00	100.0
P3	19.79	10.29	2.88	.97	
S3	33.00	7.84	5.17	1.57	108.1
P4	27.19	8.94	4.28	1.54	
S4	8.29	3.38	1.26	.46	107.2
Band(B0)	1.03	.80	.18	.14	
B1	1.51	.31	.23	.05	
B2	1.50	.33	.24	.07	
B3	14.63	3.16	2.26	.49	68.0

[a]Expresses recovery with respect to the original homogenate. (Example: % recovery for P3 (19.79 ABS U/min) = total acticity of P3 divided by total activity of H (657.53 ABS/min) times 100 gives 2.88%.)
[b]Expresses the total percentage recovery of the previous differentiation step (Example: sum for P3 (19.79 ABS U/min) and S3 (33.0 ABS U/min) divided by P2b (52.95 ABS U/min) times 100 gives 108.1%.)

Appendix Table 16. Methionine uptake by isolated jejunal and ileal BBMV from pigs fed an *Aspergillus* product[a].

	Diet							
	Control		Aspergillus					
System	Jejunum	Ileum	Jejunum	Ileum	SE	pb	pc	pd
			Conc = .1 mM					
Total	59.8	66.7	66.5	65.7	1.8	.43	.40	.29
Na^+-dep	14.9	20.2	16.8	19.8	1.2	.74	.10	.63
Na^+-indep	22.8	24.6	26.8	23.0	.8	.44	.55	.10
Diffusion	22.1	21.9	22.9	22.9	.60	.48	.91	.93
			Conc = .2 mM					
Total	105.3	119.0	118.4	122.8	3.7	.27	.24	.54
Na^+-dep	27.9	35.5	30.2	34.2	2.7	.93	.30	.75
Na^+-indep	33.1	39.8	42.4	42.9	1.4	.05	.23	.30
Diffusion	44.3	43.8	45.8	45.8	1.2	.48	.91	.93
			Conc = .4 mM					
Total	186.4	209.8	208.1	219.3	6.9	.28	.23	.66
Na^+-dep	43.6	60.0	45.0	65.8	5.0	.72	.08	.83
Na^+-indep	54.3	62.3	71.5	62.0	2.8	.16	.90	.15
Diffusion	88.6	87.6	91.6	91.5	2.4	.48	.91	.93
			Conc = .8 mM					
Total	310.2	364.9	354.8	359.5	8.0	.24	.09	.14
Na^+-dep	58.9	92.6	53.9	73.5	4.5	.20	.01	.44
Na^+-indep	74.1	97.2	117.6	102.9	6.8	.09	.77	.19
Diffusion	177.2	175.1	183.3	183.0	4.8	.48	.91	.93
			Conc = 1.6 mM					
Total	575.4	594.5	615.7	602.0	15.3	.45	.93	.60
Na^+-dep	90.9	112.8	94.8	94.1	6.5	.58	.43	.40
Na^+-indep	130.1	131.4	154.2	141.8	11.0	.45	.81	.76
Diffusion	354.3	350.3	366.6	366.1	9.7	.48	.91	.93
			Conc = 3.2 mM					
Total	980.6	1,085.8	1,130.8	1,061.0	32.5	.27	.94	.27
Na^+-dep	89.5	189.2	138.7	114.4	10.0	.53	.08	.01
Na^+-indep	182.4	196.3	258.9	214.6	20.0	.26	.71	.48
Diffusion	708.6	700.5	733.1	732.1	19.4	.48	.91	.93
			Conc = 6.4 mM					
Total	1,752.6	1,810.8	1,911.9	1,931.0	43.7	.12	.64	.79
Na^+-dep	107.9	161.4	143.9	152.1	17.6	.71	.40	.53
Na^+-indep	227.7	247.9	303.2	314.9	26.6	.20	.77	.94
Diffusion	1,417.0	1,401.0	1,464.8	1,464.0	38.7	.49	.92	.93

[a]Expressed as pmoles/mg protein/sec.
[b]Probability that a diet difference this large or larger could have occurred by chance.
[c]Probability that a site difference this large or larger could have occurred by chance.
[d]Probability that a diet*site interaction this large or larger could have occurred by chance.

Appendix Table 17. Relative contributions of Na^+-dependent, Na^+-independent, and diffusion systems to total uptake of methionine by jejunal and ileal BBMV of pigs fed an *Aspergillus* product[a].

	Diet							
	Control		Aspergillus					
System	Jejunum	Ileum	Jejunum	Ileum	SE	pb	pc	pd
			Conc = .1 mM					
Na^+-dep	25.4	30.3	25.4	30.0	1.2	.94	.07	.96
Na^+-indep	36.2	35.8	40.0	33.7	1.0	.67	.10	.15
Diffusion	38.4	33.9	34.6	36.4	.8	.67	.38	.06
			Conc = .2 mM					
Na^+-dep	25.5	29.4	25.1	27.7	1.4	.72	.29	.84
Na^+-indep	31.8	32.8	35.6	33.9	1.1	.28	.89	.55
Diffusion	42.7	37.8	44.3	38.4	1.3	.60	.13	.26
			Conc = .4 mM					
Na^+-dep	23.2	27.7	21.5	30.0	1.5	.94	.05	.52
Na^+-indep	31.8	32.8	34.4	28.3	1.1	.28	.89	.24
Diffusion	45.0	39.5	44.0	41.7	.9	.43	.10	.66
			Conc = .8 mM					
Na^+-dep	19.0	25.4	15.2	21.1	1.2	.12	.03	.91
Na^+-indep	24.3	26.1	33.4	28.0	1.7	.13	.60	.30
Diffusion	56.7	48.5	51.3	50.9	1.2	.54	.35	.12
			Con = 1.6 mM					
Na^+-dep	15.8	19.3	15.5	16.4	1.1	.48	.30	.52
Na^+-indep	22.6	20.8	25.5	23.4	1.5	.36	.53	.95
Diffusion	61.6	60.0	59.0	60.2	1.3	.62	.91	.55
			Conc = 3.2 mM					
Na^+-dep	9.4	17.4	11.8	10.3	.7	.11	.03	.01
Na^+-indep	19.0	18.1	23.3	21.0	1.5	.25	.57	.83
Diffusion	71.6	64.5	64.8	68.7	1.6	.83	.83	.67
			Conc = 6.4 mM					
Na^+-dep	6.2	8.1	7.3	7.9	.9	.75	.49	.70
Na^+-indep	13.0	14.8	16.5	17.1	1.6	.48	.83	.98
Diffusion	80.9	77.1	76.1	74.9	1.2	.19	.36	.63

[a]Percentage basis

[b]Probability that a diet difference this large or larger could have occurred by chance.

[c]Probability that a site difference this large or larger could have occurred by chance.

[d]Probabilitythat a diet*site interaction this large or larger could have occurred by chance.

Appendix Table 18 Glucose uptake by isolated jejunal and ileal BBMV of pigs fed an *Aspergillus* product[a].

	Diet							
	Control		Aspergillus					
System	Jejunum	Ileum	Jejunum	Ileum	SE	pb	pc	pd
			Conc = .075 mM					
Total	36.4	37.4	37.4	36.1	1.8	.98	.98	.75
Na^+-dep	21.0	22.8	22.9	21.3	1.6	.66	.62	.99
Na^+-indep	9.6	9.3	9.0	9.3	.4	.70	.99	.70
Diffusion	5.8	5.4	5.6	5.6	.2	.93	.58	.58
			Conc = .15 mM					
Total	58.1	59.7	63.8	63.7	3.0	.62	.70	.89
Na^+-dep	29.4	30.7	37.3	33.4	2.5	.31	.80	.62
Na^+-indep	17.2	18.1	15.4	19.1	.6	.76	.08	.29
Diffusion	11.5	10.8	11.1	11.1	.3	.93	.58	.58
			Conc = .3 mM					
Total	103.5	111.7	110.4	123.3	4.9	.43	.66	.71
Na^+-dep	48.8	54.5	55.4	56.0	4.4	.66	.74	.78
Na^+-indep	31.7	35.6	32.8	45.1	2.5	.31	.13	.42
Diffusion	23.1	21.6	22.2	22.2	.6	.93	.58	.58
			Conc = .6 mM					
Total	221.2	216.8	211.3	225.8	9.8	.90	.69	.75
Na^+-dep	101.8	97.4	102.0	109.0	7.2	.69	.93	.70
Na^+-indep	73.3	76.2	64.9	72.4	2.5	.26	.33	.67
Diffusion	46.1	43.3	44.5	44.4	1.2	.93	.57	.58
			Conc = 1.2 mM					
Total	369.9	373.7	379.7	387.3	15.4	.71	.86	.96
Na^+-dep	140.3	144.4	163.9	147.8	11.4	.80	.57	.67
Na^+-indep	137.3	142.6	126.9	150.6	6.1	.93	.26	.47
Na+-dep	92.2	86.5	88.9	88.9	2.5	.93	.57	.58
			Conc = 2.4 mM					
Total	663.3	662.3	618.9	666.6	23.6	.68	.63	.62
Na^+-dep	248.8	251.0	216.7	246.5	18.3	.63	.67	.71
Na^+-indep	230.0	238.3	224.4	242.3	8.7	.97	.47	.79
Diffusion	184.4	173.0	177.9	177.8	4.9	.93	.57	.58
			Conc = 4.8 mM					
Total	1,161.0	1,118.1	1,071.4	1,195.5	38.8	.94	.62	.62
Na^+-dep	354.8	346.8	307.0	339.6	24.8	.59	.81	.69
Na^+-indep	438.3	425.2	408.8	500.5	14.0	.43	.18	.09
Diffusion	368.8	346.0	355.6	355.5	9.9	.93	.58	.58
			Conc = 9.6 mM					
Total	1,846.6	1,767.0	1,721.6	1,822.0	47.4	.91	.73	.25
Na^+-dep	394.3	378.8	337.0	340.8	29.3	.43	.93	.88
Na^+-indep	714.6	696.2	673.3	770.1	27.8	.78	.50	.32
Diffusion	737.7	692.1	711.3	711.1	19.8	.93	.58	.58

[a]Expressed as pmoles/mg protein/sec.
[b]Probability that a diet difference this large or larger could have occurred by chance.
[c]Probability that a site difference this large or larger could have occurred by chance.

Appendix Table 19. Relative contributions of Na^+-dependent, Na^+-independent, and diffusion systems to total uptake of glucose by jejunal and ileal BBMV of pigs fed an *Aspergillus* product[a].

	Diet							
	Control		Aspergillus					
System	Jejunum	Ileum	Jejunum	Ileum	SE	pb	pc	pd
			Conc = .075 mM					
Na^+-dep	57.7	59.9	60.2	58.7	2.4	.51	.47	.67
Na^+-indep	26.4	25.4	24.6	25.6	1.2	.54	.80	.49
Diffusion	15.9	14.7	15.3	15.6	.7	.80	.52	.37
			Conc = .15 mM					
Na^+-dep	49.4	51.2	58.5	50.9	2.4	.26	.38	.23
Na^+-indep	30.4	30.5	24.1	30.5	1.4	.52	.49	.51
Difuusion	20.2	18.3	17.4	18.6	.8	.99	.33	.87
			Conc = .3 mM					
Na^+-dep	46.3	48.6	50.2	46.5	2.3	.70	.67	.96
Na^+-indep	31.1	31.7	29.7	38.2	2.0	.63	.21	.27
Diffusion	22.6	19.7	20.1	20.7	.7	.89	.25	.37
			Conc = .6 mM					
Na^+-dep	46.0	44.3	46.3	46.3	1.6	.87	.66	.66
Na^+-indep	33.1	35.3	31.3	32.5	1.1	.19	.72	.91
Diffusion	20.8	20.4	22.4	21.3	.7	.61	.36	.93
			Conc = 1.2 mM					
Na^+-dep	37.1	38.0	41.2	36.8	1.7	.68	.62	.45
Na^+-indep	37.6	38.0	34.3	38.8	1.4	.64	.38	.47
Diffusion	25.3	24.0	24.5	24.4	.9	.92	.68	.71
			Conc = 2.4 mM					
Na^+-dep	37.1	37.6	33.2	35.6	1.7	.40	.68	.79
Na^+-indep	34.9	35.7	36.4	36.8	1.4	.65	.83	.95
Diffusion	28.0	26.7	30.4	27.6	.9	.36	.26	.67
			Conc = 4.8 mM					
Na^+-dep	29.9	30.6	27.8	27.8	1.3	.35	.89	.90
Na^+-indep	38.0	37.7	37.4	41.7	1.0	.41	.34	.29
Diffusion	32.1	31.7	34.8	30.5	.8	.64	.16	.25
			Conc = 9.6 mM					
Na^+-dep	21.4	21.4	19.3	18.7	1.5	.36	.80	.80
Na^+-indep	38.7	38.7	38.0	42.3	1.0	.75	.50	.51
Diffusion	39.9	39.9	42.7	39.0	1.1	.75	.35	.36

[a]Percentage basis

[b]Probability that a diet difference this large or larger could have occurred by chance.

[c]Probability that a site difference this large or larger could have occurred by chance.

[d]Probability that a diet*site interaction this large or larger could have occurred by chance.

Appendix Table 20. Methionine uptake by isolated jejunal and ileal BBMV from lambs fed an *Aspergillus* product[a].

	Diet							
	Control		Aspergillus					
System	Jejunum	Ileum	Jejunum	Ileum	SE	pb	pc	pd
			Conc = .1 mM					
Total	66.9	63.4	58.2	68.1	.7	.57	.36	.06
Na^+-dep	33.0	30.0	28.0	36.0	1.5	.86	.41	.08
Na^+-indep	16.3	15.1	12.3	12.1	1.0	.09	.72	.81
Diffusion	17.6	18.3	17.9	20.0	.6	.43	.28	.55
			Conc = .2 mM					
Total	113.8	112.9	103.8	117.3	2.8	.62	.27	.30
Na^+-dep	52.9	52.4	46.1	57.2	2.8	.83	.37	.33
Na^+-indep	25.7	24.0	21.9	20.0	1.8	.31	.28	.99
Diffusion	35.2	36.5	35.8	40.1	1.7	.41	.27	.56
			Conc = .4 mM					
Total	184.5	192.0	176.4	203.9	4.6	.85	.08	.26
Na^+-dep	72.0	76.2	73.1	91.4	3.5	.26	.13	.49
Na^+-indep	42.1	42.8	31.8	32.4	3.4	.15	.93	.76
Diffusion	70.4	73.0	71.5	80.1	2.5	.42	.28	.56
			Conc = .8 mM					
Total	313.5	309.3	307.4	336.8	7.2	.47	.40	.26
Na^+-dep	102.8	101.9	108.1	122.3	5.2	.24	.53	.49
Na^+-indep	69.9	61.3	56.3	54.3	5.1	.33	.62	.76
Diffusion	140.8	146.0	143.0	160.2	4.9	.42	.28	.56
			Conc = 1.6 mM					
Total	524.5	534.9	492.6	570.4	11.0	.94	.07	.15
Na^+-dep	134.4	147.0	137.3	156.8	8.4	72	.36	.84
Na^+-indep	108.4	95.9	69.3	93.1	10.0	.31	.78	.38
Diffusion	281.7	292.0	286.0	320.5	9.9	.42	.28	.55
			Conc = 3.2 mM					
Total	975.2	961.6	854.3	978.5	26.0	.33	.30	.21
Na^+-dep	187.5	164.0	151.9	190.0	12.9	.86	.78	.26
Na^+-indep	224.4	213.6	130.3	147.6	20.7	.07	.94	.74
Diffusion	563.3	584.0	572.1	640.9	19.8	.42	.28	.56
			Conc = 6.4 mM					
Total	1,502.0	1,618.4	1,468.0	1,715.1	38.5	.70	.03	.41
Na^+-dep	88.6	161.0	169.9	236.5	13.1	.08	.02	.93
Na^+-indep	286.8	289.4	154.1	196.9	24.2	.04	.65	.69
Diffusion	1,126.5	1,168.0	1,144.1	1,281.5	39.6	.42	.28	.56

[a]Expressed as pmoles/mg protein/sec.
[b]Probability that a diet difference this large or larger could have occurred by chance.
[c]Probability that a site difference this large or larger could have occurred by chance.
[d]Probability that a diet*site interaction this large or larger could have occurred by chance.

Appendix Table 21. Relative contributions of the Na^+-dependent, Na^+-independent, and diffusion systems to total uptake of methionine by jejunal and ileal BBMV of lambs fed an *Aspergillus* product[a]

	Diet							
	Control		Aspergillus					
System	Jejunum	Ileum	Jejunum	Ileum	SE	pb	pc	pd
			Conc = .1 mM					
Na^+-dep	48.3	45.6	47.6	51.4	1.4	.36	.84	.25
Na^+-indep	24.4	23.6	19.5	16.8	1.5	.07	.58	.76
Diffusion	27.4	30.8	32.9	31.8	.9	.10	.53	.22
			Conc = .2 mM					
Na^+-dep	45.6	44.6	43.1	47.1	1.7	1.00	.66	.46
Na^+-indep	22.5	21.1	19.7	16.2	1.6	.26	.47	.74
Diffusion	32.0	34.3	37.2	36.7	1.0	.07	.64	.49
			Conc = .4 mM					
Na^+-dep	38.3	37.8	40.8	43.5	1.2	.12	.66	.54
Na^+-indep	22.7	22.5	17.2	15.9	1.8	.11	.84	.88
Diffusion	39.1	39.7	41.9	40.6	1.2	.43	.87	.68
			Conc = .8 mM					
Na^+-dep	32.5	32.1	35.0	35.1	1.4	.33	.95	.94
Na^+-indep	22.2	19.0	17.0	15.9	1.6	.20	.50	.74
Diffusion	45.3	48.9	48.0	49.0	1.2	.56	.33	.59
			Conc = 1.6 mM					
Na^+-dep	25.5	27.0	27.8	27.4	1.3	.62	.84	.71
Na^+-indep	21.1	17.1	13.8	16.2	1.8	.83	.28	.39
Diffusion	53.4	55.9	58.4	56.4	1.5	.39	.93	.49
			Conc = 3.2 mM					
Na^+-dep	18.9	17.2	17.8	18.9	1.0	.90	.88	.50
Na^+-indep	22.5	21.4	15.0	15.1	1.8	.08	.89	.88
Diffusion	58.6	61.4	67.2	66.0	2.0	.13	.84	.63
			Conc = 6.4 mM					
Na^+-dep	5.9	9.9	11.6	13.5	.7	.003	.04	.44
Na^+-indep	19.8	18.0	10.3	11.5	1.6	.02	.93	.64
Diffusion	72.1	78.2	75.0	85.2	1.8	.34	.45	.90

[a]Percentage basis

[b]Probability that a diet difference this large or larger could have occurred by chance.

[c]Probability that a site difference this large or larger could have occurred by chance.

[d]Probability that a diet*site interaction this large or larger could have occurred by chance.

Appendix Table 22. Glucose uptake by isolated jejunal and ileal BBMV from lambs fed an *Aspergillus* product[a].

	Diet							
	Control		Aspergillus					
System	Jejunum	Ileum	Jejunum	Ileum	SE	pb	pc	pd
			Conc = .075 mM					
Total	127.5	46.9	87.2	41.3	5.3	.05	.0001	.12
Na^+-dep	105.6	27.8	68.9	23.3	4.6	.05	.0001	.11
Na^+-indep	15.5	14.4	12.6	13.6	1.0	.36	.99	.58
Diffusion	6.4	4.8	5.7	4.4	.4	.55	.11	.87
			Conc = .15 mM					
Total	182.3	67.2	126.8	62.8	7.8	.08	.0001	.13
Na^+-dep	143.9	35.6	94.4	33.3	7.2	.09	.0001	.12
Na^+-indep	25.6	22.1	20.9	20.8	1.9	.44	.63	.67
Diffusion	12.8	9.5	11.5	8.8	.9	.55	.11	.87
			Conc = .3 mM					
Total	237.4	103.3	178.0	101.3	.6	.16	.0001	.18
Na^+-dep	163.9	45.6	120.2	46.0	9.0	.25	.0001	.24
Na^+-indep	47.9	38.6	34.9	37.7	2.9	.26	.59	.32
Diffusion	25.6	19.1	22.9	17.6	1.7	.55	.11	.87
			Conc = .6 mM					
Total	327.3	165.6	233.1	157.8	12.3	.06	.0002	.10
Na^+dep	195.9	53.7	112.9	55.1	9.8	.06	.0002	.05
Na^+-indep	80.2	73.8	74.4	67.5	5.7	.61	.57	.99
Diffusion	51.2	38.1	45.8	35.1	3.4	.55	.11	.87
			Conc = 1.2 mM					
Total	453.9	273.4	336.7	244.7	15.1	.03	.0004	.17
Na^+-dep	197.4	61.9	107.5	48.8	34.7	.02	.0001	.06
Na^+-indep	154.1	135.2	137.6	125.6	10.9	.56	.49	.88
Diffusion	102.4	76.2	91.7	70.3	6.9	.55	.11	.87
			Conc = 2.4 mM					
Total	661.2	456.3	532.3	440.1	23.5	.15	.007	.25
Na^+-dep	159.2	60.0	108.6	65.6	12.6	.39	.02	.28
Na^+-indep	297.1	243.7	240.4	234.0	18.9	.40	.44	.55
Diffusion	204.9	152.6	183.3	140.5	13.7	.55	.11	.87
			Conc = 4.8 mM					
Total	1,063.9	719.9	853.2	811.6	41.7	.49	.04	.09
Na^+-dep	192.3	76.9	123.8	65.0	17.8	.28	.03	.44
Na^+-indep	461.8	337.8	362.8	465.5	42.5	.87	.91	.21
Diffusion	409.8	305.2	366.6	281.1	27.4	.55	.11	.87
			Conc = 9.6 mM					
Total	1,643.9	1,308.3	1,435.8	1,308.9	66.6	.49	.04	.11
Na^+dep	212.5	66.3	178.3	74.4	17.3	.71	.003	.55
Na^+-indep	611.8	631.7	524.2	672.3	64.8	.86	.53	.47
Diffusion	819.5	610.3	733.2	562.2	54.2	.55	.10	.95

[a]Expressed as pmoles/mg protein/sec.
[b]Probability that a diet difference this large or larger could have occurred by chance.
[c]Probability that a site difference this large or larger could have occurred by chance.
[d]Probability that a diet*site interaction this large or larger could have occurred by chance.

Appendix Table 23. Relative contributions of Na^+-dependent, Na^+-independent, and diffusion systems to total uptake of glucose by jejunal and ileal BBMV of lambs fed an *Aspergillus* product[a].

	Diet							
	Control		Aspergillus					
System	Jejunum	Ileum	Jejunum	Ileum	SE	pb	pc	pd
			Conc = .075 mM					
Na^+-dep	82.4	55.6	78.9	51.0	2.4	.41	.0002	.92
Na^+-indep	12.3	33.0	14.4	36.1	1.7	.47	.0002	.89
Diffusion	5.3	11.5	6.7	12.9	1.2	.58	.03	.99
			Conc = .15 mM					
Na^+-dep	77.7	49.5	73.3	46.9	2.7	.55	.0002	.90
Na^+-indep	14.7	34.7	17.3	35.9	2.3	.69	.0008	.89
Diffusion	7.6	15.6	9.1	17.2	1.7	.67	.03	.98
			Conc = .3 mM					
Na^+-dep	67.6	42.2	67.1	39.7	2.6	.77	.0002	.85
Na^+-indep	21.1	38.7	20.0	39.7	2.1	.97	.0004	.76
Diffusion	11.3	15.9	13.0	20.6	1.9	.73	.06	.94
			Conc = .6 mM					
Na^+-dep	58.7	31.0	48.0	32.8	2.6	.40	.007	.24
Na^+-indep	25.0	45.0	32.4	43.0	2.3	.56	.005	.23
Diffusion	16.3	24.0	19.6	24.1	2.2	.71	.19	.73
			Conc = 1.2 mM					
Na^+-dep	42.6	22.0	31.3	19.9	1.9	.11	.001	.26
Na^+-indep	34.0	49.7	41.6	50.0	2.7	.48	.05	.52
Diffusion	23.3	28.3	27.1	30.1	2.4	.57	.42	.84
			Conc = 2.4 mM					
Na^+-dep	23.2	12.8	19.8	16.0	2.0	.99	.10	.43
Na^+-indep	45.4	54.1	45.2	50.6	2.8	.75	.23	.78
Diffusion	31.4	33.2	35.0	33.5	2.8	.73	.98	.77
			Conc = 4.8 mM					
Na^+-dep	17.9	10.6	13.8	8.8	1.7	.40	.09	.74
Na^+-indep	42.9	46.3	42.9	53.9	3.7	.62	.34	.62
Diffusion	39.2	43.1	43.3	37.3	3.4	.91	.88	.48
			Conc = 9.6 mM					
Na^+-dep	13.1	5.2	12.8	5.8	1.2	.96	.008	.92
Na^+-indep	36.3	48.3	36.2	47.7	3.6	.99	.13	.84
Diffusion	50.6	46.6	51.0	46.5	3.8	1.00	.60	.83

[a]Percentage basis

[b]Probability that a diet difference this large or larger could have occurred by chance.

[c]Probability that a site difference this large or larger could have occurred by chance.

[d]Probability that a diet*site interaction this large or larger could have occurred by chance.

Appendix Table 24 Example of Statistical analysis of Variance for Alkaline Phosphatase Enrichment by Isolated Intestinal BBMV from Lambs Fed *Aspergillus* Product.

General Linear Models procedure

Dependent Variable: ALP Enrichment

Source	DF	Sum of Squares	Mean Squares	F Value	Pr > F
Model	18	15.4037	0.8558	1.58	0.3216
Error	5	2.7013	0.5403		
Corrected Total	23	18.1049			

R-Square	C.V	Root MSE	ALP Enrichment Mean
0.8508	13.1000	0.7350	5.6106

Source	DF	Type I SS	Mean Squares	F Value	Pr > F
Diet	1	2.6947	2.6947	4.99	0.0758
Pair	5	4.1920	0.8384	1.55	0.3207
Site	1	3.7131	3.7131	6.87	0.0470
Diet*Pair	5	2.6900	0.5380	1.00	0.5018
Diet*Site	1	0.7873	0.7873	1.46	0.3814
Pair*Site	5	1.3265	0.2653	0.49	0.7731

Source	DF	Type III SS	Mean Squares	F Value	Pr > F
Diet	1	2.6947	2.6947	4.99	0.0758
Pair	5	4.1920	0.8384	1.55	0.3207
Site	1	3.7131	3.7131	6.87	0.0470
Diet*Pair	5	2.6900	0.5380	1.00	0.5018
Diet*Site	1	0.7873	0.7873	1.46	0.3814
Pair*Site	5	1.3265	0.2653	0.49	0.7731

Appendix Table 25 Example of Statistical Analysis of Variance for Isolated Mucosal Weight from Lambs Fed *Aspergillus*Product.

General Linear Models procedure

Dependent Variable: Mucosal weight of Jejunum

Source	DF	Sum of Squares	Mean Squares	F Value	Pr > F
Model	6	16977.96	2829.66	6.15	0.0325
Error	5	2301.80	460.36		
Corrected Total	11	19279.77			

R-Square	C.V	Root MSE	Mucosal weight Mean
0.8806	10.8332	21.4560	198.06

Source	DF	Type I SS	Mean Squares	F Value	Pr > F
Diet	1	2255.02	2255.02	4.90	0.0778
Pair	5	14722.94	2944.59	6.40	0.0314

Source	DF	Type III SS	Mean Squares	F Value	Pr > F
Diet	1	2255.02	2255.02	4.90	0.0778
Pair	5	14722.94	2944.59	6.40	0.0

Appendix Table 26. Example of Statistical Analysis of Variance for Na^+-dependent Uptake from Lambs Fed *Aspergillus* Product.

General Linear Models procedure
Conc = .075 mM
Dependent Variable: Na^+-dep uptake

Source	DF	Sum of Squares	Mean Squares	F Value	Pr > F
Model	8	30393.99	3799.25	7.44	0.0005
Error	15	7657.30	510.49		
Corrected Total	23	38051.30			

R-Square	C.V	Root MSE	Na+-dep uptake mean
0.7988	40.0634	22.59	56.40

Source	DF	Type I SS	Mean Squares	F Value	Pr > F
Diet	1	2555.03	2555.03	5.01	0.0409
Tissue	1	22891.34	22891.34	44.84	0.0001
Pair	5	3389.31	677.86	1.33	0.3052
Diet*Tissue	1	1558.32	1558.32	3.05	0.1010

Source	DF	Type III SS	Mean Squares	F Value	Pr > F
Diet	1	2555.03	2555.03	5.01	0.0409
Tissue	1	22891.34	22891.34	44.84	0.0001
Pair	5	3389.31	677.86	1.33	0.3052
Diet*Tissue	1	1558.32	1558.32	3.05	0.1010

Appendix Table 27. Example of Statistical Analysis of Variance for Average Daily Gain from Pigs Fed *Aspergillus* Product.

General Linear Models procedure

Dependent Variable: ADG 2

Source	DF	Sum of Squares	Mean Squares	F Value	Pr > F
Model	13	65563.01	5043.31	1.25	0.3668
Error	10	40308.33	4030.83		
Corrected Total	23	105871.35			

R-Square	C.V	Root MSE	ADG 2 Mean
0.619271	20.20	63.4888	314.27

Source	DF	Type I SS	Mean Squares	Value	r > F
Diet	1	1047.70	1047.70	0.26	0.6212
Group	1	23796.00	23796.00	5.90	0.0355
Pair(Group)	10	39347.79	3934.78	0.98	0.5148
Diet*Group	1	1371.51	1371.51	0.34	0.5726

Source	DF	Type III SS	Mean Squares	F Value	Pr > F
Diet	1	1047.70	1047.70	0.26	0.6212
Group	1	23796.00	23796.00	5.90	0.0355
Pair(Group)	10	39347.79	3934.78	0.98	0.5148
Diet*Group	1	1371.51	1371.51	0.34	0.5726

VITA

Insurk Jang, son of Jaehae Jang and Haesoon Jung, was born on July 22, 1962 in Taegue, Korea. He graduated Tae Ryun high school in February 1981. Insurk continued his education at the Department Animal Science at the Yeung Nam University in Korea. He received a Master's Degree in Animal Science majoring in Animal Nutrition. He then had military service for 6 months as a second lieutenant, called Master's officer. He moved to the United States of America and began studies leading to Ph.D. degree in the College of Agriculture and Life Sciences at Virginia Polytechnic Institute and State University, Blacksburg VA in 1989. He completed the requirements for the Doctor of Philosophy Degree in Animal Science in October, 1993.

Insurk Jang is a member of the American Society of Animal Science and the Korean Society of Animal Science.

Insurk Jang