EFFECTS OF DIETARY GLUTAMINE ON SOW AND LITTER PERFORMANCE AND NURSERY PIG PERFORMANCE AND INTESTINE GROWTH

By

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DISSERTATION TITLE

Effects of Dietary Glutamine on Sow and Litter Performance and Nursery Pig Performance and Intestine Growth

BY

Steven J. Kitt

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EFFECTS OF DIETARY GLUTAMINE ON SOW AND LITTER PERFORMANCE AND NURSERY PIG PERFORMANCE AND INTESTINE GROWTH

Steven J. Kitt (Ph D)

University of Nebraska, 2003

Adviser: Phillip S. Miller

There are marked changes in the gastrointestinal structure and function of pigs after weaning. Specifically, villous (absorption cells) atrophy and crypt (cells that replace villous cells) hyperplasia occur following weaning. This change in growth of the small intestine is generally associated with a decrease in digestive and absorptive capacity and vulnerable to environments stresses.

Therefore, nutrients that improve the growth stasis and intestinal degeneration after weaning may be important in the diet during or after weaning. Glutamine has been proposed to be the primary substrate to promote intestinal growth. It was hypothesized that glutamine may improve the growth performance via improving the intestinal function and (or) immune response after weaning. Three experiments were designed test our hypothesis via providing glutamine (considered a dietary nonessential amino acid) to the weaned pig. Experiment 1 involved feeding a low concentration of glutamine in two diet types. Pigs

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exhibited slightly better efficiency of gain during d 14 to 21 after weaning. Experiment 2 was designed by using all purified ingredients to discern whether glutamine is important after an imposed immune challenge. Pigs fed glutamine after an immune challenge maintained their feed intake, weight gain, efficiency of gain, and intestinal growth compared to non-immune challenged pigs. Experiment 3 was designed to make a practical approach to feeding glutamine to weanling pigs. Sows were fed increased glutamine, milk was sampled and contained greater glutamine than controls. However, growth performance was slightly less in progeny from sows fed glutamine than controls. However, intestinal growth (villus height) was greater in pigs fed glutamine. Taken together, these data suggest that glutamine is an important dietary nutrient during an immune challenge, but feeding glutamine prior to an immune challenge via sow milk may be detrimental for overall body growth.

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May God bless you all.

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Literature Review

Introduction

Currently, the approach to feeding weanling pigs is not a sound scientific process. Unlike all other stages of production in swine, and as described by Pettigrew (2000), nutritional programs are often centered on feeding minimum amounts of ingredients rather than minimum amounts of nutrients. Specific dietary ingredients elicit a growth response that cannot be accounted for by current knowledge of the nutrients within the ingredient. This response is primarily due to the improvement in growth performance (i.e., feed intake, daily gain) observed when high "quality" diets (Whang et al., 2000) containing ingredients such as spray-dried animal plasma (Ermer et al., 1994), blood meal, fish meal, and milk products are offered to the weanling pig. Therefore, nutrient requirement experiments that employ the use of these ingredients may observe "ingredient" effects and consequently may confound the "nutrient" effects observed. Conversely, experiments that do not use these ingredients may limit feed intake and growth of the pigs and inherently decrease the nutrient requirements of the pig because the genetic potential is not maximized. Therefore, experiments that lead to better

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understanding of the biology of the newly weaned pig will improve upon the understanding of the nutrient needs of the newly weaned pig. Additionally, it is important to better define the nutrient(s) that are responsible for eliciting improved growth responses associated with specific ingredients. However, it is even more important to improve upon our understanding of the relationships between nutrition, growth, and health if we are to feeding practices of weanling pigs.

Intestinal Changes of the Newly Weaned Pig

Weaning is an extremely stressful event in the pig's life. The pig is taken away from its dam, moved to a new environment, commingled with pigs from other litters, and switched from a liquid-milk diet to a dry diet. Pluske et al. (1997) stated that there is little doubt that low voluntary food intake and the associated poor growth after weaning are major limitations to enhanced efficiency in pig production. During this time, the small intestine undergoes extreme physical and functional changes. Villus atrophy and crypt hyperplasia have been extensively cited (for review see Pluske et al, 1997) as physical changes observed in weaned pigs and it is suggested that these changes are potentially causative in digestive, absorptive,

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and immune limitations of the weaned pig. In fact, Pluske et al. (1996) have shown that villus height is correlated to empty body weight gain and can explain 47% of the variation in empty body weight gain the first five days after weaning. Therefore, it appears that improvements in and (or) maintaining the integrity of the small intestine immediately post-weaning may improve overall growth of the pig. The cause(s) of intestinal morphologic changes after weaning is of some debate but several factors have been suggested.

Potential Causes of Changes in Intestinal Morphology

Apoptosis: A "normal" change after weaning?: It is possible that intestinal degeneration via apoptosis is normal and even biologically advantageous after weaning. Apoptosis ("programmed cell death") is quite different from necrotic cell death. Inflammation due to the rupture of membranes of the cell causing phagocytosis of the cell is one major distinctive feature of necrosis that is not observed in apoptosis. The events observed during apoptosis are cell shrinkage, disappearance of microvilli, detachment from neighboring cells, and chromatin condensation. Ultimately cells separate into intact

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membrane bound bodies and are phagocytized by like-neighboring cells (Que and Gores, 1996).

The quantitative biological significance of apoptosis in the literature is vague. Que and Gores (1996) described apoptosis as physiological cell death that has evolved to remodel tissue, maintain tissue homeostasis, remove senescent cells, and delete cells with impairable genetic damage. Similarly, Lavin and Waters (1993) suggested that apoptosis occurs at a low rate in most healthy adult mammalian tissue, where it compliments mitosis in steady state kinetics.

Apoptosis is initiated by growth factor withdrawal, DNA damaging agents, anticancer drugs, reactive oxygen species, UV and γ radiation, pro-inflammatory cytokines (TNF α, IL 1, IL 16, bacterial toxins, and interferon γ), and cell detachment from the local tissue (Tarnawski and Szabo, 2001). Certainly, the healthy intestine is prone to reactive oxygen species, pro-inflammatory cytokines, and cell detachment, and possibly growth factor withdrawal (in situations of decreased blood flow). Interestingly, the lack of nutritional factors, glutamine (Papaconstantinou et al., 1998) and butyrate (from nutrient fermentation in the colon; Hass et al., 1997) have also been implicated in

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heightened apoptosis. These initiation factors begin a signal transduction cascade which can be further propagated by specific cellular apoptotic signals such as: altered calcium homeostasis, free radical generation, p53 dependent pathway cascade, or mitochondrial dysfunction (and release of cytochrome C) (Mattson et al., 2001). The continued cellular signal of "death" activates effector caspases, other proteases, or repress "life genes". Finally, the cell is at the point of no return and apoptosis has begun. The intestine is no exception to cell death via apoptosis. Hall et al. (1994) suggested that apoptosis probably accounts for a high portion of cell loss in the intestine. Due to a high rate of proliferation in the crypt region and migration of epithelial cells towards the tip of the villus, cell death via apoptosis would be expected to balance the high rate of proliferation. Frish and Francis (1994) first demonstrated detachment of the epithelial cell from the epithelial matrix induces apoptosis and termed this version of apoptosis "anoikis". Moss and Holt (1996) agreed that apoptosis occurs at the tip of the villus by extrusion into the lumen of the intestine. Iwanaga et al. (1993) suggested that the matrix holding the epithelial cells is phagocytized by macrophages. Whether these

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extruded cells detach prior to or after death is unknown. Additionally, apoptosis has been shown to take place in the crypt at the level of the stem cell and is believed to be a process to regulate the number of cells entering the cell cycle destined for the villus (Watson and Pritchard, 2000). Apoptosis is rarely seen along the length of the villus. Although the aforementioned studies are fairly convincing, it is important to remember that cell culture systems have innate disadvantages because these cell lines have been altered to live longer and are not in their native environment. Therefore, the apoptotic rate and mechanism may be altered. Models developed in vivo have been used to more accurately study apoptosis. Gene knock out mice (Watson and Pritchard, 2000) have revealed that apoptosis is induced by different genes in different tissues under different conditions. For example, Table 1 reveals that the Bcl2 gene products are responsible for repressing apoptosis in the small intestine but not the large intestine. Another example is that p53 gene products induce apoptosis under gamma radiation but not during blood flow restriction. Using similar methodology as described by Watson and Pritchard (2000), Moss et al. (1995) suggested that the apoptotic rate of the colon is much

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greater than that of the small intestine (see Table 2). Therefore, the location (e.g., small intestine versus colon) and type of stressors can dramatically influence the apoptotic rate of the intestinal tract. From these data, it is unreasonable to ascribe "blanket statements" regarding apoptosis in the intestinal tract.

Apoptosis is a normal physiological phenomenon in the small and large intestine. The quantitative significance is unknown and therefore the emphasis placed on apoptosis as a "typical" mechanism for cell regeneration in the intestinal tract is reasonable and may depend on the type and severity of the stressor as well as the site of the intestinal tract.

Table 1. Genetic determinants of apoptosis in mouse intestinal epithelium. Adapted from Watson and Pritchard, 2000.

		Bcl2		
Stimulus	P53	Small intestine	colon	Bax
Spontaneous	-	-	+	-
Gamma radiation	++	-	++	-
5-Fluorouracil	++	+	++	+/-
Ischemia-reperfusion	-	+	ND	ND

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Table 2. Genetic expression of Bcl2 related proteins and apoptotic rate in small intestine and colon. Adapted from Moss et al. (1995).

	Ileum	Colon
Apoptotic cells	1.6 %	38 %
Bcl2	-	++
Bax	+	++
BclxL	+	+++
BclxS	-	-
Mcl 1	+	+

Feed Intake. Although dietary energy density (Cole et al., 1971), sex, temperature, and disease status (Lewis and Southern, 2001) are thought to be the primary feed intake regulation controls for growing pigs, these factors have less reproducible effects on weanling pigs. By combining several data sets, LeDividich and Herpin (1994) concluded that maintenance energy intake is not met until five days following weaning. The lack of feed intake may directly or indirectly contribute to post weaning intestinal changes. For example, restrictively feeding (60% of ad libitum; Nunez et al., 1996) formula-fed pigs has been shown to decrease the villus height and increase crypt depth of the small intestine. Intravaneous feeding of rats has shown similar results (Goodlad et al., 1992), suggesting that the presence of luminal substrate and (or) physical contact is necessary for intestinal cell proliferation. Using a different approach, Pluske et al. (1996) offered ewe milk

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to 28-d old weaned pigs every 2 h. Five days after weaning, pigs that had consumed ewe milk had maintained villus height and crypt depth compared to preweaned pigs. This experiment also showed that dry matter intake was correlated (R = 0.60) with villus height. The availability of substrate to enterocytes or other intestinal cells could explain the maintenance of villus height in this experiment. However, it is possible that this experiment was confounded by the methodology because nutrients or other factors that are unique to milk could have influenced the outcome. These factors will be discussed in "Factors Found in Milk".

However, Kelly et al. (1991) used a cereal-based diet and fed via gastric intubation and observed increased villus height and increased crypt depth compared to pigs fed ad libitum (and therefore, less feed intake). A lack of feed intake has been shown to promote inflammation via an increase in inflammatory T cell numbers and expression of matrix metalloproteinase stromelysin (McCracken et al., 1999). These increases in DNA expression were observed in conjunction with reduction in villus height and have been suggested as evidence that weaning anorexia appears to play a significant role in the morphological changes of the

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small intestine. Overall it appears that improved feed intake in weanling pigs improves intestinal morphology; however, mechanisms that define feed intake (or lack of feed intake) in weanling pigs are not fully understood.

Stress and Catabolic Hormones. Although stress associated with weaning is commonly referenced in the literature as a potential cause of disruption in intestinal morphology, little documented evidence exists relating stress to intestinal measurements. In pigs diagnosed with "wasting pig syndrome", plasma cortisol and cortisol binding capacity were less than concentrations found in age-matched, healthy pigs (Albinsson and Anderson, 1990). Although reduced villus height and crypt cell proliferation rate was less in "wasting" pigs, the lack of relationship with plasma catabolic hormone concentration suggests that cortisol is not a causative factor in intestinal atrophy. However, other catabolic hormones may contribute to intestinal disturbances after weaning. McCracken et al. (1995) observed a diet independent increase in glucagon, interleukin-I, and fibrinogen. These metabolic factors were suggested to be partially responsible for decreased growth rate. However, in this study pigs weaned to a liquid milk diet had greater villus height:crypt depth

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ratio compared to pigs fed a cereal based diet regardless of metabolite concentrations. This suggests that intestinal lumen substrate or other factors intrinsic to milk present in the milk based diet may impact intestinal cell proliferation to a higher degree than systemic measures of stress. Alternatively, it could be argued that the diet type (i.e., liquid vs. dry diet; or deleterious compounds in soybean protein isolates) confounded the results observed in the small intestine. The most conclusive and carefully designed experiment to study the effects of "weaning stress" was conducted by Van Beers-Schreurs et al. (1998). In their experiment pigs weaned and fed sow milk had similar villus height as compared to unweaned (suckling) pigs, suggesting that weaning stressors (besides the stress of a change in diet form or type) do not play a significant role in compromising intestinal morphology.

Imbalance of or Lack of Specific Cytokines. An imbalance or lack of specific cytokines may contribute to altered intestinal morphology and function. Injury to the intestinal epithelial cells is often the result of inflammation due to the immune response following antigen invasion or attachment. Because the epithelial lining of

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the intestine is thought to be the first line of defense against consumed antigens, it is obvious that lesions in this tissue must be mended quickly. Localized healing as well as other biological processes are controlled by growth factors and cytokines. Cytokines are defined as (Bendtzen, 1994) polypeptide or glycopeptide signaling molecules that act at extremely low concentrations that are important mediators of infectious, immunoflammatory reactions, or hormone-like end results. Cytokines generally act locally much like autocrine or paracrine functions of growth factors and hormones. Until recently it was presumed that epithelial cells acted only as a passive defense barrier. However, it has been discovered that epithelial cells synthesize cytokines and growth factors important in the immune response and maintenance of its own integrity (Perdue and McKay, 1994). Cytokines synthesized by intestinal epithelial cells include (but are not limited to) transforming growth factor (TGF) α, TGF β 1, tumor necrosis factor (TNF) α, interleukin (IL) 1, IL 1 β, IL 1 receptor antagonist, IL 6, and IL 8. Synthesis of these cytokines is dependent on stimulus of other growth factors, cytokines, or compounds and is summarized in Table 3. Ogra et al. (1999) stated that although epithelial cells produce

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many cytokines, they do not synthesize the classic T cell cytokines IL 2, IL 4, and IL 5.

Table 3. Cytokines synthesized by epithelial cells of the intestine. Adapted from Perdue and McKay (1994) and text from Ogra et al. (1999)

Cytokine	Type	Synthesized in response to	Note
IL 6	Inflammatory	IL 1 β, TGF β, TNF α, cholera toxin	Increased in colon cancer patients
IL 8	Inflammatory	IFN γ, TNF α, IL 6, EGF, E. coli LPS, Salmonella attachment	
TNF α	Inflammatory	Inflammatory cytokines, LPS	
MCP 1	Inflammatory	IL 1 β, TNF α	"chemokine"
ENA 78	Inflammatory	IL 1 β, TNF α	"chemokine"
IL 1	Inflammatory	?	Induces proliferation
IL 7	Regulatory	?	Induces proliferation
IL 10	Regulatory	?	
IL 15	Regulatory	?	
SCF	Regulatory	?	
TSH	Regulatory	?	
IL 8 mRNA		?	Not confirmed in vivo

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No data is available to shed light on the quantitative significance of the type or sources of cytokines for repair of the intestine after injury. Generally, other cell types that secrete cytokines responsible for proliferation may be just as influential as epithelial cells themselves. At the present time, one can only "match" synthesis of specific

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cytokines with each cell type and presume that the specific cell may contribute to proliferation.

Although important for eventual healing of epithelial cells, enhancing proliferation of epithelial cell growth (via up-regulation of specific genes for expression of growth factors and cytokines) is not quick enough (e.g., hours to days) to seal the barrier between the lumen of the intestine and the animal. Therefore, a mechanism that is responsive to epithelial damage is required to maintain the epithelial integrity at least until genetic machinery (i.e ., transcription, translation, and protein synthesis) can be amplified to rebuild it. The cells respond by flattening and migrating into the lesion to close (Ogra et al., 1999) within minutes to hours after tissue damage. This process has been called "restitution" and is controlled by cytokines. Dignass and Podolsky (1993) showed that TGF β is the central controlling cytokine directing other cytokines and growth factors (TGF α, epidermal growth factor, interleukin 1 β, and interferon γ) immediately following epithelial cell injury. Apparently, integrins and fibronectin are also important during restitution. Integrins are matrix binding cell surface proteins that are necessary for cell migration and

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