ABSTRACT

CABRERA, RAFAEL ARISTIDES. Improving Early Postnatal Piglet Health, Growth and Development: Effects of Supplemental Milk Replacer, IgG, Glutamine and Glutamic Acid. (Under the direction of Dr. Jack Odle and Dr. Adam J. Moeser.)

The immunity acquired by neonatal piglets during the suckling period is critical for their protection against diseases beyond the colostrum period. The influence of maternal age on the immune competence of her progeny is known to be important but has been more extensively studied in dairy cattle than pigs. Moreover, how the piglets' long-term growth and viability beyond the colostrum period are influenced by the amount of time they spend nursing their mother after removing the effect of birth weight has never been studied. A total of 1,034 pigs produced by breeding PIC sows to 2 different PIC terminal sires were used to create 3 distinct weaning weight populations so that post-weaning growth to 125 kg could be studied: Sow reared (**SR**) (N = 367) for 20 d, sow reared for 14 d (**14W**) (N = 330) and sow reared for 2 d (**2W**) (N = 337). We found that there was a linear relationship ($P < 0.05$) between weaning weight and ADG in the post-nursery phase of growth. Two major conclusions were drawn from this experiment: 1) a weaning weight of less than 5.0 kg weaning weight imposes the greatest marginal loss in production output for a 20-d weaning, and that (2) lactation length positively influences long-term growth, composition of growth and viability of the progeny.

In experiment two, we surveyed serum immunoglobulin G (IgG) content of 2 d old piglets in a commercial sow farm and the effect of birth order, birth weight, sow colostrum IgG concentration, and sow parity on piglet pre-weaning survival rate. We found that sow

colostrum IgG concentration and birth order can account for 10% of the variation of pig IgG concentration and that piglets with less than 1,000 mg/dl IgG serum concentration and birth weight of 0.9 kg at birth had low survival rate when compared to their heavier siblings. Piglet IgG concentration and birth weight had the greatest effects (57% and 64% respectively) of any of the variables measured on % survival at weaning.

In experiment thee, we determined the effect of time and feeding state on IgG absorption, intestinal morphology, and expression of IgG receptors in the first 24 h post-birth. Twenty newborn pigs were fitted with umbilical arterial catheters and orally-gavaged with 32 ml defatted colostrum per kg of body weight either at birth (0 h) or at 12 h post-birth under either fed (milk replacer) or fasted (saline solution) conditions (n = 5/group). A fifth reference group (n=5), was euthanized at birth. There was no interaction between the time (age) of colostrum gavage (0 vs. 12 h) and nutritional state (fed vs. fasted). Serum IgG increased progressively with time, reaching peak concentrations at 8 h post-gavage. Piglets given colostrum at 0 h had higher ($P < 0.05$) overall IgG absorption and higher ($P < 0.05$) villi height than those gavaged at 12 h post birth. Abundance of FcRn transcript was lower ($P = 0.006$) in pigs euthanized at birth compared with those gavaged at 0 h and killed at 12 h of age.

In the last experiment, we investigated the impact of adding creep feed, glutamine, and glutamate plus glutamine (Aminogut) to pre- and post-weaning diets. Litters (N=120) were allotted to four treatments during 14-21 d of lactation: 1) Non-creep fed (NC, n=45); 2) creep fed control diet (CFCD, n=45); 3) creep fed 1% GLN (CFGLN, n=15); or 4) creep fed .88% AG (CFAG, n=15). No effects of creep feeding on intake, weaning weight or mortality were

detected (P>0.25). After weaning, the NC and CFCD groups were sub-divided into three groups (n=15 each), receiving either a control nursery diet (NC-CD, CFCD-CD) or a diet supplemented with either GLN (NC-GLN, CFCD-GLN) or with AG (NC-AG, CFCD-AG). The litters creep fed diets containing GLN or AG also were supplemented with those amino acids in the nursery diets (CFGLN-GLN, CFAG-AG). Pigs receiving GLN in both pre- and post-weaning diets (CFGLN-GLN) had the best feed conversion (feed/gain) among all the treatments for the first thee-week period ($P = 0.056$). The NC-AG group had ($P = 0.02$) the greatest feed intake among all treatments in the last three weeks of the study. CFGLN-GLN, CFCD-GLN and Sow Reared (SR) had the greatest ($P < 0.05$) villi height and the greatest ($P < 0.0001$) number of cells proliferating (PCNA). We also found that pigs creep fed with a diet supplemented with AminoGut and also fed with a post-weaning diet supplemented with AminoGut (CFAG-AG) had the deepest ($P < 0.01$) crypts among all the treatments. Sow reared (SR) pigs showed the greatest ($P < 0.0001$) intestinal absorption capacity for xylose and mannitol. There was no significance difference among the others treatments on the absorption of these sugars and their maltase activity.

Improving Early Postnatal Piglet Health, Growth and Development: Effects of Supplemental Milk Replacer, IgG, Glutamine and Glutamic Acid

by
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DEDICATION

I want to dedicate this work to four persons in my life:

To my mother **Argentina Dolores Rosario.** She has always showed the greatest determination to raise her family together. She changed hospital beds walking 10 miles every day in the heat of the summer in the Dominican Republic. She raised all of us with dignity and never gave up. From her I learned that we must do that we think is impossible. I will always love you mom.

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Without any of you, my life would not have been complete. I love you with all my heart.

BIOGRAPHY

Rafael A. Cabrera was born January 20th, 1965 in the Dominican Republic, to Rafael Cabrera and Argentina Rosario. He received his High School diploma (graduated best in the entire island) and Bachelor in Science (Cum Laude) from the Dominican Republic. In December of 1986 (prior to his Bachelor's graduation) he was in route to the United States to start his Master in Science. He enrolled at Iowa State University for an intensive English course for 6 months. After successfully completing the course, he went on to the University of Missouri-Columbia to pursue his Master in Science where he graduated Magna Cum Laude in Animal Nutrition. Upon graduation, Rafael was invited to be a Research Fellow at the University of Wageningen (1992-1993). He later came to North Carolina to work as a Research Associate at Carroll's Foods under the supervision of Dr. Ronald Nimmo for 2 years. Then he worked as Production Swine Unit Manager for Goldsboro Hog Farms managing a 2,000 sow farrow-to-wean. In 1997, Rafael decided to join PIC (Pig Improvement Company, Inc.) in Franklin, KY as Director of Sales for Central America for one year (1997-1998) and later became part of PIC Nutrition Technical Services under the supervision of Dr. Dean Boyd. During that capacity, the PIC Nutrition Team developed the nutritional requirements for all PIC genetic lines. After 5 years with PIC, Rafael joined Ralco Nutrition, Inc; (Marshall, MN) as Director of Technical Services for Latin America. During his stay at Ralco, Rafael decided to start his PhD in the fall of 2007. He held his position at Ralco for 8 years until last year when he joined Huvepharma USA as Director of Technical Services. In this capacity, Rafael is responsible for validating Huvepharma's products with field research, developing new products and providing technical services to existing customers and prospects. Rafael has

been married to Maria Dolores for the last 21 years and they have two college boys: Camilo Cabrera and Gabriel Cabrera. Camilo is Junior at Stanford University majoring in Chemical Engineering and Gabriel is a freshman at Santa Clara University majoring in Business.

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LITERATURE REVIEW

PRODUCTION OF SOW COLOSTRUM AND MILK

The importance of the maternal influence of the sow on the nutrition and immunity of her progeny cannot be emphasized enough. After parturition and during the suckling period, the sow provides the main nutrient source for the suckling piglets therefore understanding the quantity and quality of this nutrient source becomes extremely relevant for the survival of the neonatal piglets. In the last decade more research has been concentrated on early weaning and rearing of piglets by artificial means. The knowledge generated by these practices has allowed us to use the pig as model to study human nutrition and immunology (Reeds and Odle, 1996). Like many farm animals, the pig is born with low energy reserves (Mellor and Cockburn, 1986) and without immune protection (Gaskins, 1998). Colostrum provides these two components as well as other important growth factors such as EGF, IGF-I, IGF-II and Insulin (Odle et al., 1996). Colostrum also plays an important role in the development of the gastrointestinal tract of the piglet (Xu et al., 2002).

Sow milk output becomes a great limitation in order for neonatal pigs to maximize full potential for body growth (Harrell et al., 1993; Hartmann et al., 1984, Zijlstra et al., 1996). Greater genetic selection pressure has been placed in litter size over sow milk output. This milk output is greatly influenced by the suckling demand imposed by the number (Auldist et al., 1998) and size (King et al., 1997) of piglets. Auldist and co-workers (2000) reported that the increased sow milk yield associated with a larger litter is mainly a function of an increased number of functional glands, whereas the increased milk yield in response to heavier piglets is

associated with increased production per gland. Milk production from individual glands may also be influenced by suckling frequency. The typical suckling frequency interval varies from 30 to 70 min for individual sow during the first week of lactation (Jensen et al., 1991).

The production of colostrum is quite variable among sows and the factors affecting this variability are not well known (Farmer and Quesnel, 2009). Colostrum secretion occurs during the key transition period from the anabolic metabolism of gestation to the catabolic metabolism of lactation (Bauman and Curie, 1980). Sow colostrum production has been estimated to vary from 2.5 to 5 kg over 24 h for a litter of 8 to 12 piglets. This roughly accounts for 360 g/pig/d intake of colostrum. This calculation is consistent with the results published by Devillers (2004) wherein colostrum intake of sow-reared piglets ranged from 212 to 373 g/kg of birth weight. However colostrum intake of bottle-fed piglets during the first 24 h after birth had a voluntary intake exceeding 450 g/kg of birth weight (Harrell et al. 1993). The amount of colostrum available per piglet decreases by 22 to 42 g per each additional piglet born (Devillers et al., 2007). The mean efficiency of conversion of milk to gain has been found to be 4.5 g of milk per 1 g of pig gain (Lewis et al., 1978).

Other authors have shown that this limitation extends beyond the 24 h period. Cabrera et al., (2010) weaned pigs as early as 2 d of age and compared their growth to their sow-reared counterparts up to weaning. The early-weaned pigs reached an average body weight during 18 d (total age of 20 d) of supplemental milk replacer of 8.75 kg vs. 6.49 kg for sow-reared piglets (or 393 g/d vs. 272 g/d respectively). However their post-weaning growth was followed until slaughter, and they grew (from 20 d of age until slaughter) slower (785 g/d vs. 816 g/d respectively) and were not significantly heavier than their sow-reared counterparts

(123.9 kg vs. 123.5 kg respectively). This is probably due to compromise health and/or digestive upset. Unlike other studies (Mahan, 1993; Mahan and Lepine, 1991) where pigs with different birth weights are tracked until slaughter, this is was an impressive finding since we removed the birth effect among the treatments. This study also has a profound implication in terms of the characteristics of the composition of the sow's milk vs. commercial milk replacers. Sow's milk has proven to have benefits beyond the colostrum period.

Spencer and others (2003) observed greater growth rate when they early weaned pigs at d 14 and fed milk replacer. They observed growth of 681 g/d from d 14 until d 19. Milk replacer was the only source of nutrients and the room temperature was kept at 30 C. They did not follow the grow-finish period performance in those milk-fed pigs.

Devillers et al., (2007) reported no effects of age, BW or duration of parturition on colostrum yield. However they reported that there was a tendency for greater production of colostrum for second and third parity sows when compared to primiparous or older sows. Mahan (1998) also reported a linear decrease in colostral fat content as parity advanced with the largest decline occurring in parity 1 to parity 2.

It is also important to stress the effects of nutrition on sow colostrum. The general tendency during the last 4 wks of gestation is to increase daily feed intake since during that time ¾ of the growth of the newborn pig takes place. However this practice can have detrimental effect if not managed properly. It is generally accepted that overfeeding during gestation has a negative impact on mammogenesis due to excessive fat deposition in sows (Farmer and Sorensen, 2001). On the other hand, feed restrictions at the end of gestation may

only have a small detrimental effect on colostrum production because of the sow's body size and large energy reserves.

COMPOSITION OF SOW MILK

The production of milk by the sow starts during the final h of gestation with the production of colostrum (highly pigmented and viscous colostral secretion in the mammary gland). The most comprehensive study conducted to determine the true composition of sow milk during lactation has been conducted by Klobasa and co-workers (1987). They studied the amounts of total solids (TS), fat, lactose, total protein, and total whey protein, their interrelationships and the changes in these interrelationships throughout lactation in 25 German Landrace sows (Table 1).

Table 1. Composition of sow milk throughout lactation (adapted from Klobasa et al., 1987).

Stage	TS (%)	Fat (%)	Lactose (%)	Total Protein (%)	Whey Protein (%)	NPN (%)
0 h	25.6	5.0	3.1	15.7	14.3	0.11
6 h	22.7	4.8	3.4	13.0	10.9	0.11
12 h	18.4	4.9	4.1	8.8	7.0	0.09
18 h	17.7	5.2	4.4	7.3	5.6	0.09
24 h	17.3	5.6	4.6	6.4	4.6	0.10
48 h	18.6	6.5	4.8	6.4	3.9	0.12
72 h	19.0	6.7	5.2	6.1	3.7	0.12
5 d	18.4	6.5	5.5	5.5	3.2	0.13
7 d	18.3	6.7	5.6	5.4	3.0	0.12
14 d	18.2	6.4	5.9	5.1	2.7	0.13
21 d	18.7	6.6	5.8	5.2	2.8	0.14
28 d	18.1	6.1	5.8	5.4	2.8	0.14
35 d	17.6	5.5	5.7	5.7	3.0	0.15
42 d	17.0	5.3	5.4	6.0	3.1	0.15

In this study all sows farrowed within 4 d and they found that in the first 6 h of lactation colostrum total solids (TS) and protein contents were higher, while fat and lactose contents were lower than in mature milk. Decreased total protein decreased whey protein contents and increased fat and lactose content with unchanged TS levels, indicate transition from colostrum to mature milk. The high protein content is largely due to immunoglobulin. No influence of parity and litter size on milk composition was observed in this study.

These results are very consistent with those reported by Mateo et al., 2004. They found in twelve multiparous sows (Landrace x Yorshire x Duroc) that Total Milk Solids (TMS) decreased from 26.7% on d 0 to 23.1% on d 3. The TMS further decreased to 19.3% on d 7 but then remained constant at 18.2%, 18.8% and 19.2% on d 14, 21 and 28, respectively. The concentration of CP decreased from 16.6% in colostrum to 7.7%, 6.2%, 5.5%, 5.7% and 6.3% in milk collected on d 3, 7, 14, 21 and 28, respectively. They also found (not measured by Klobasa and others, 1987) that in colostrum 5'UMP represented 98% of all 5'monophosphate nucleotides, and in milk, 5'UMP accounted for 86 to 90% of all nucleotides, regardless of the day of lactation. This indicates that TMS and CP values for sow's milk has remained virtually the same in the last 15 years regardless the changes observed in genetics, housing systems, level of productivity and diet formulation.

The effects of nutrition on sow colostrum composition have been widely studied. The addition of fat in late pregnancy has been shown to increase total lipid concentration in colostrum (Boyd et al., 1981; Coffey et al., 1982; Jackson et al., 1995; Chiston et al., 1999; Heo et al., 2008), lactose content and IGF-1 concentrations (Averette et al., 1999). A reduction of protein for gestating sows from 16 to 13% did not seem to change colostral fat

content (Mahan, 1998). However the increase of dietary lysine intake (8.0 g/kg instead of 6.0 g/kg) in late gestation increased total solid and protein contents in colostrum in sows (Heo et al., 2008; Yang et al., 2008). Bland and others (2001) and Pinelli-Saavedra and co-workers (2008) have increased dietary vitamin concentration during gestation and saw an increased of vitamin concentrations in porcine colostrum. A more complete of summary of the immune-modulating effects of active components on immunoglobulin concentrations in porcine colostrum is presented in Table 2.

Table 2. Effects of active components on immunoglobulin contents of porcine colostrum (adapted from Farmer and Quesnel, 2008).

Treatment	Duration[1]	Effect	Reference
Conjugated Linoleic Acid	8 d	↑IgG	Bontempo et al., 2004
Phytogenic Feed Additive (Essential Oils)	1 wk	↑IgG	Wang et al., 2008
Plant Extract	1 wk	↓IgG, IgA	Ilsley et al., 2003
Mannan Oligosaccharides	2 wk	↑IgM, = IgG, IgA	Newman and Newman, 2001
Mannan Oligosaccharides	3 wk	↑IgG, IgA, IgM	O'Quinn et al., 2001

[1]Duration of treatment before parturition.

Besides energy, protein and immunoglobulins, sow's colostrum and milk have some growth factors (Table 3, adapted from Odle et al., 1996) which have an enormous influence on the intestine development of the suckling neonate. Most of these growth factors are acid-

stable and have no problem reaching the small intestine intact avoiding degradation by gastric secretion (Odle et al., 1996).

Table 3. Concentrations (µg/L) of growth factors in sow's colostrum and milk (Adapted from Odle et al., 1996).

Growth Factors	Colostrum	Mature Milk	Reference
Epidermal Growth Factor (EGF)	1,500 ± 525	160 to 240	Jaeger et al., 1987
Insulin-like Growth Factor (IGF-I)	39 ± 22	11.4 ± 1.4	Donovan et al., 1994
Insulin-like Growth Factor (IGF-II)	82.3 ± 57.5	16.8 ± 5.6	Donovan et al., 1994
Insulin	12.3 ± 3.3	1.6 to 3.3	Jaeger et al., 1987

PREWEAN MORTALITY IN NEONATAL PIGLETS AND TRANSFER OF PASSIVE IMMUNITY

Despite increased improvements in management practices, the mortality of newborn piglets remains unacceptably high at 15% (USDA, 2007). Large swine integrators with more than 100,000 sows will have even higher mortality. This high mortality results from a combination of both improved sow genotype and consequently yielding larger litter size and reduction in labor in order to reduce production costs. Crushing by the dam accounts for more than 40% of piglet's death, while starvation and digestive upset account for more than 30% (USDA, 2007).

During the first 24 h it is critical that piglets get their portion of colostrum and consequently their passive immunity from the dam. During the suckling period, the neonate

will not synthesize antibodies on its own because it receives ample circulating antibodies from the dam. The average half-life of these antibodies (i.e. IgG is 9.5 d) is between 1 to 2 weeks. After that the neonate will start making antibodies when exposed to antigens. When an antigen first appears, it is recognized by Antigen Presenting Cells (APC) such as dendritic cells which in turn present the antigen to T-cells (thymus derived cells) and from those cells the message finally arrives to the B-cells (bone-marrow derived cells). The stimulated B-cells then undergo mitosis and blast formation. The blast cells are the cells which actively produce and secrete antibodies. The whole process from when antigens are presented until antibodies are formed is between 3 to 4 days (Stormont, 1972).

In calves, IgG molecules are not present in the blood-stream of newborn because they cannot cross the placenta during pregnancy. In a study conducted by the USDA (1993), blood samples of 2,177 calves were collected between 24 to 48 h of age and it was determined that IgG represented nearly 90% of all the immunoglobulins transferred to calves in colostrum. The report also noted that the level of IgG that provides adequate protection is around 1,000 mg/dl. This is consistent with the results we reported in chapter 3. IgG molecules cannot cross the placenta in pigs during pregnancy and therefore accounts for the lack of IgG in the neonate's blood prior to birth (See Table 4, adapted from Redman, 1979). Neonatal piglets that suffer failure of passive transfer of maternal immunoglobulins (passive immunity) may be at increased risk for diseases. After the pigs are weaned, they start developing their own immunity (active) since maternal antibodies start disappearing.

Table 4. Transmission of Maternal Antibody (adapted from Redman, 1979).

Species	Placental Type	Tissue Layers	Prenatal	Postnatal
Horse, Pig	Epitheliochorial	6	-	+++
Cow, Sheep	Syndesmochorial or Epitheliochorial	6	-	+++
Dog	Endotheiochorial	4	+	++
Rat, Mouse	Haemochorial	4	+	++
Rabbit, Guinea pig	Haemochorial	2	+++[a]	-
Man, Monkey	Haemochorial	2	+++[a]	-

[a]Yolk sac route.

Sow colostrum's multi-faceted immunological value for the suckling piglet has been extensively studied (Speer et al., 1959; Payen and Marsh, 1962; Leece and Morgan, 1962; Hardy, 1965; Butler, 1979; Werhahn et al., 1981; Klobasa et al., 1981, 1986). The immunoglobulins of porcine colostrum provide the major antimicrobial protection against microbial infections and confer a passive immunity to the newborn calf until its own immune system matures. IgG constitutes the predominant (see Table 5) colostral Ig in porcine (Klobasa et al., 1987, Wilson, 1974) and in bovine as well (Korkonen et al., 2000).

Table 5. Concentration (mg/ml) of IgG, IgM and IgA in sows' whey during lactation (Adapted from Klobasa et al., 1987).

Stage	IgG	IgM	IgA
0 h	95.6	9.1	21.2
6 h	64.8	6.9	15.6
12 h	32.1	4.2	10.1
18 h	21.6	3.2	6.7
24 h	14.2	2.7	6.3
48 h	6.3	2.7	5.2
72 h	3.5	2.4	5.4
5 d	1.8	2.1	5.2
7 d	1.5	1.8	4.8
14 d	1.0	1.5	4.8
21 d	0.9	1.4	5.3
28 d	0.8	1.4	5.6
35 d	0.8	1.7	7.8
42 d	0.8	1.8	9.4

Others researchers (Porter, 1969; Curtis and Bourne, 1971) have reported values of 80% IgG, 15% IgA and 5% IgM of the total immunoglobulins present in porcine colostrum. Of the total protein content in porcine colostrum, 60 to 70% is comprised by immunoglobulins (Svendsen et al., 1971). IgG half life has been reported to be 9.7 days (Frenyo et al., 1981), 10 days (Klobasa et al., 1981) and 14 days (Curtis and Bourne, 1973). After 10 days of age, piglets are capable of producing their immunoglobulins and most of the immunoglobulins produced are of the IgM class with lesser quantities of IgA produced (Allen and Porter, 1973).

The most complete study determining the absorption and synthesis of maternal IgG, IgA and IgM in piglet's serum levels for the first eight weeks of life has been conducted by Klobasa and co-workers (1981). Group **A** (94 animals) was maintained with the sow. Subgroups of piglets were withdrawn for 0 to 24 h before returning to the sow. Group **B** (45 animals) was reared in an automatic rearing device on bovine colostrum and milk and animals

were given various quantities of purified swine IgG during the first day of life. Finally, 41 naturally reared piglets that failed to survive in their natural environment were recorded. In group **A,** peak serum immunoglobulin levels were reached 12 h after beginning of suckling. Absorption of immunoglobulins was directly related to the length of time the piglets were removed from the sow, the decrease being caused by the decrease of immunoglobulin levels in colostrum. In piglets deprived of colostrum, only IgM was present in serum at birth but all thee isotopes were present by one week. Administration of 3.5 g of swine IgG had the same effect on synthesis of all thee isotypes as was observed in the naturally reared animals. Nearly all of the 41 piglets in the group which failed to survive were shown to have absorbed only 10 to 50% as much immunoglobulins as their age-matched surviving controls in the first 12 h after suckling.

THE ROLE OF THE MHC CLASS I-RELATED PORCINE NEONATAL Fc RECEPTOR IN THE UPTAKE AND TRANSPORT OF IMMUNOGLOBULIN G

Immunoglobulins (Ig) are composed of two heavy and two light chains each of which contains an NH_2-terminal antigen-binding variable domain and a COOH-terminal constant domain responsible for the effector functions of antibodies. The COOH-terminal domains of Ig heavy chains form the Fc region involved in triggering cellular activities though its interactions with specific receptors, called Fc Receptors (FcR). On the basis of sequence and structural similarities, Ig super family member domains are divided into three sets: C1, C2 and V-like (Williams and Barclay, 1988). The C1 set includes antibody constant and topologically equivalent domains. FcRn (Fc Receptor neonatal) is the only FcR that is a member of the Ig

super family by virtue of containing constant or C1- set domains. It is called “neonatal” because its expression can be found in the first few days post-birth and declines with age.

Fridman (1989) has described FcR for all Ig classes in mice and humans: IgG (FcγR), IgE (FcϵR), IgA (FcαR), IgM (FcμR) and IgD (FcδR) and also reported that these receptors are found on all cell types of the immune system. These types of FcR react with IgG.

FcγRI is a high-affinity receptor binding IgG monomers whereas FcγRII and FcγRIII are low affinity receptors (Fridman, 1991). Two receptors involved in transepithelial transport of Ig have been characterized by the cloning of their respective cDNA’s. Mostov and co-workers (1984) reported that the poly-Ig receptor (poly-Ig-R) mediates transcellular transport of polymeric IgA and IgM into external secretions. This receptor is composed of five Ig-like external domains and a TM region followed by a short IC tail. FcγR mediates the transport of maternal IgG intact across cellular barriers between mother and offspring, avoiding degradation during transport (Simister and Mostov, 1989; McCarthy et al., 2000). This transport is located at the duodenum and jejunum level where enterocytes express a surface membrane receptor (Borvak et al., 1998) able to bind the Fc portion of the IgG molecule and to facilitate transcytosis of these immunoglobulins (Van de Perre, 2003).

FcγR and FcRn (neonatal Fc receptor) proteins are specific for IgG. FcRn is a type I membrane glycoprotein that acts as a specific receptor for the IgG isotype (Jones and Waldman, 1972). Values have been reported of the equilibrium association constant (K_A) range from ~ 2 x 10^7 M^{-1} for the interaction of monomeric IgG with isolated microvilli membranes from neonatal rat intestine (Wallace and Rees, 1980) to ~ 5 x 10^7 M^{-1} for the interaction between purified soluble rat FcRn and monomeric IgG (Raghavan et al., 1995b).

This high affinity ensures that FcRn can efficiently transport IgG. A striking difference between FcRn and the others FcRs is that FcRn is a heterodimer. Its light chain is β_2 (Simister and Rees, 1985) microglobulin which is the same light chain that is associated with class I major histocompatability complex (MHC) molecules (Figure 1a). The heavy chains of both FcRn and class I MHC molecules consist of thee extracellular domains, α1, α2, and α3 (Simister and Mostov, 1989a; Bjorkman and Parham, 1990), followed by a transmembrane region and a short cytoplasmic sequence. Simister and Mostov (1989a) reported that although the extracellular region of FcRn and class I MHC molecules exhibits low but significant sequence similarity (22-30% identity for the α1 and α2 domains; 35-37% for the α3 domain), the transmembrane and cytoplasmic regions of the two types of proteins show no detectable sequence similarity. MHC class I molecules have no reported function as Ig receptors, instead they bind and present short peptides to T cells (Townsend and Bodmer, 1989). Burmeiser and co-workers (1994a) reported: a) the crystal structure of rat FcRn confirmed that the overall fold is very similar to that of class I MHC molecules, such that the α1 and α2 domains form a platform of eight anti-parallel β-strands topped by two long α-helices and the α3 and β_2m domains are β-sandwich structures similar to antibody or C1-set domains (figure 1a) and b) a dimer of FcRn heterodimers mediated by contacts between α3 and β_2m domains was observed in these different crystal forms of FcRn. Because of the 2:1 binding stoichiometry between FcRn and IgG (Huber et al., 1993), it was suggested that the FcRn dimer could represent a receptor dimer induced by IgG binding (Burmeister et al., 1994a,b). Gastinel and others (1992) reported that in the absence of IgG, FcRn is predominantly monomeric in solution at

μM concentrations and therefore it was assumed that the high protein concentrations required for crystallization could induce formation of the FcRn dimer in the absence of IgG.

The main role of the protein FcRn (Fc Receptor neonatal) is to transfer IgG molecules from the mother to the fetus (in humans) or from the apical membrane of the epithelial cells to the blood stream (in swine). This mechanism is the only one by which mammalian neonates acquire humoral immunity to antigens encountered by the mother. This is critical because the neonate's immune system is not fully developed. Maternal IgG from the milk is bound by the receptor, transported across the gut epithelium in a process named transcytosis (Mostov and Simister, 1985), and then released to the blood stream from the basolateral membrane (Rodewald and Kraehenbuhl, 1984). Intestinal epithelial cells show a pH of between 6.0-6.5 in the apical membrane and a neutral pH between 7.0-7.5 elsewhere. This pH difference facilitates the efficient unidirectional transport of IgG, because FcRn binds IgG at pH 6.0-6.5 in suckling rats (Jones and Waldman, 1972) and mice (Mackenzie and Keeler, 1984) but not at neutral or higher pH (Rodewald and Kraehenbuhl, 1984; Simister and Mostov, 1989a; Raghavan et al., 1995a). This shows that FcRn is pH dependent. In addition to these transport functions, current evidence suggests that FcRn is the catabolic receptor that controls the lifetime of Igs in serum. It has been proven that mutant Fc fragments that show impaired FcRn binding in vitro and that are deficient for transcytosis in neonatal mice also have abnormally short serum half lives (Kim et al, 1994; Popov et al., 1996). FcRn differs most strikingly from the pig Immunoglobulins Receptor (pIgR) in its ability to transport its ligand in both the apical to basolateral and basolateral to apical direction. This statement is true in rats however it is yet to be proven in others species such as neonatal piglets.

In conclusion, most FcR are released in cell supernatants and circulate in biological fluids as immunoglobulin binding factors (IBF) generated either by cleavage at the cell membrane or by splicing of FcR transmembrane exon. Their involvement has been postulated in several diseases and monoclonal antibodies to FcR are beginning to be used in therapeutics, particularly to target cytotoxic effector lymphocytes and monocytes to tumor cells. Raghavan and Bjorkman summarized the ability of receptors for the Fc domain of Ig molecules to form crucial components of the immune system in a) facilitating specialized transport of antibody molecules to regions of the body where they are needed, b) forming the crucial link between antigen binding by Igs effector responses such as inflammation and the regulation of antibody production and finally c) controlling the lifetime of Ig molecules in serum.

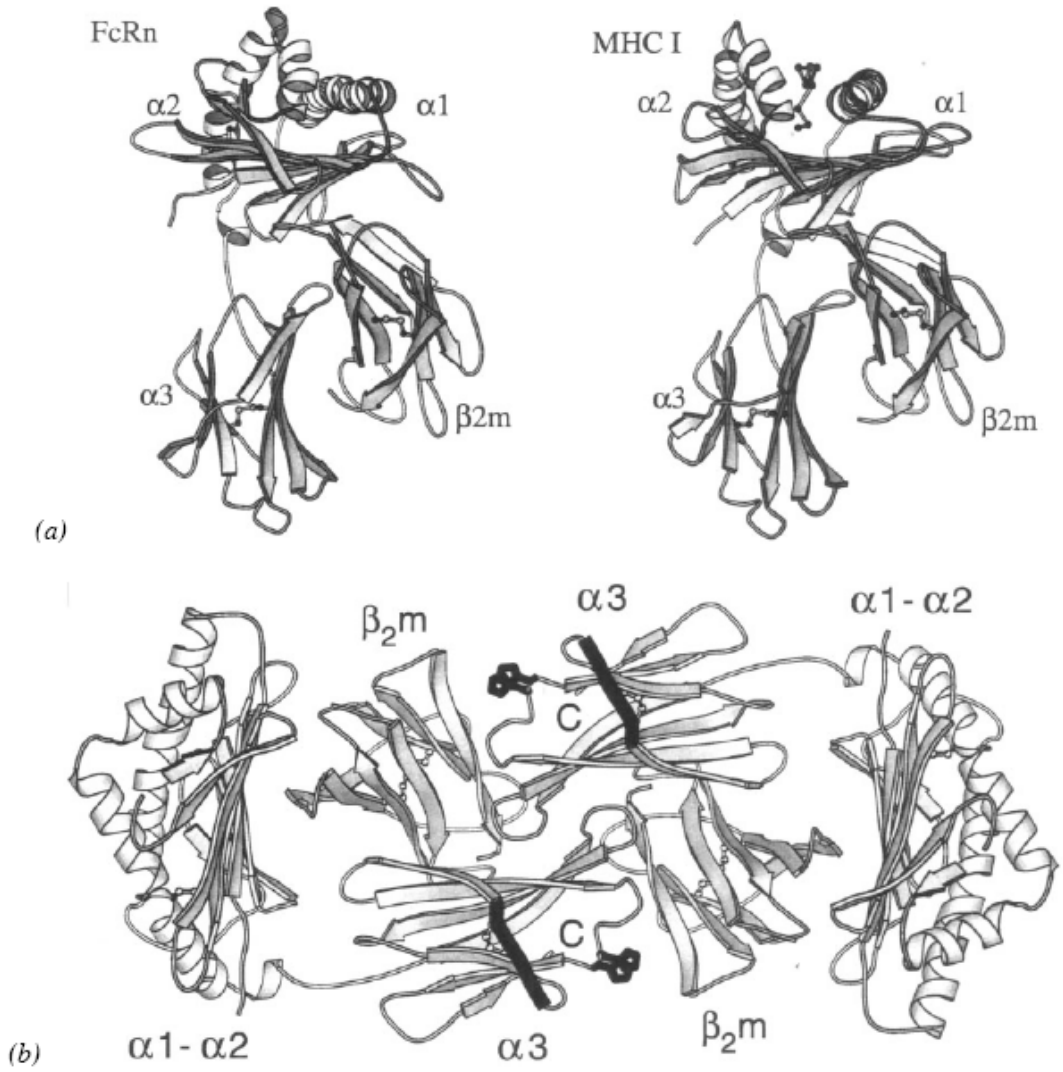


Figure 1. FcRn Structure (adapted from Raghavan and Bjorkman, 1996).

INTESTINAL MACROMOLECULAR TRANSMISSION AND CLOSURE IN NEONATAL PIGS

The prenatal pig intestine is similar to the neonatal pig intestine in that colostrum stimulates both the macromolecule absorption and the cessation of macromolecule uptake (intestinal closure) (Sangild et al., 1999).

Neonatal mortality is very high in farm animals (approximately 10%) and disease resistance is greatly influenced by an adequate passive immunization just after birth. In piglets, foals, calves and lambs, the intestinal absorption of immunoglobulins from their mother's colostrum occurs mainly by a non-specific endocytosis of macromolecules, but the details of the absorption process, and the mechanisms regulating its cessation after 1-2 days of colostrum exposure, remain poorly understood (Sangild (2003). In both normal and 'compromised' (premature, growth-retarded, hypoxic, lethargic) newborn farm animals, the intestinal capacity to absorb macromolecules is influenced by both diet- and animal-related factors. Thus, macromolecule uptake is severely reduced in response to premature birth and when macromolecules are to be absorbed from diets other than species-specific colostrum. On the other hand, fetal growth retardation, in vitro embryo production, or stressful birth processes are unlikely to reduce the ability of the intestine to absorb immunoglobulins from colostrum. More knowledge about the diet- and animal-related factors affecting intestinal immunoglobulin uptake will improve the clinical care of 'compromised' newborn farm animals.

Uptake of colostrum just after birth is essential to stimulate intestinal growth and function, and in many species, including pigs, colostrum also provides immunological

protection via the absorption of immunoglobulin G (IgG). Jensen and co-workers (2001) investigated the intestinal growth, IgG absorptive capacity and enzyme activities in newborn pigs in response to different diets. Newborn piglets were bottle-fed porcine colostrum (PC), bovine colostrum (BC), porcine plasma (PP), porcine milk (PM), bovine colostrum containing porcine plasma (BCP) or a milk replacer (MR) every 3 h (15 mL/kg) for up to 2 d. Bovine serum albumin (BSA) was added to the diets as a macromolecule marker. The percentage of absorbed BSA just after birth was highest for piglets fed the PC diet (30-50%), lower for those fed the BC and BCP diets (23-30%) and lowest for the PP, PM and MR diet-fed piglets (7-20%, $P < 0.05$ relative to those fed colostrum). Porcine IgG was absorbed more efficiently than bovine IgG. Intestinal closure occurred earlier in MR and BCP piglets (within 12 h after birth) than in PC pigs. At 2 d of age, intestinal mucosal weight (+120% increase from birth) and villus morphology were similar in the PC, BCP and MR groups. All 3 groups also had increased aminopeptidase A activity compared with values at birth (+100% increase). Compared with PC pigs, the BCP group had higher sucrase and maltase activities (+50% and +200%, respectively) and lower aminopeptidase N activity (-50%, $P < 0.05$). Similarly, MR pigs showed elevated sucrase activity (+40%) and lowered maltase, lactase and aminopeptidase N activities (-20% to -50%, $P < 0.05$) compared with PC pigs. They concluded that porcine and bovine colostrum contain factors that stimulate the intestinal endocytotic and enzymatic capacity in newborn pigs. A milk replacer can produce normal gut growth, but may be inefficient in mediating normal macromolecule transport and disaccharidase activity. Bovine colostrum mixed with porcine plasma proteins may be a useful substitute for porcine colostrum in artificial rearing of newborn pigs.

Rundell and Lecce (1971) reported that intestinal cell epithelial cell turnover and closure are unrelated in species other than the rat. They concluded that the turnover time of porcine intestinal epithelium to be about five days and that intestinal closure (uptake and transport) occurred before the initial cell population was replaced. Others have reported the process of incorporation of IgG into the neonatal's circulation stops when the enterocytes are unable to discard the absorbed IgG though the lateral membranes. Moon (1971) supported Rundell and Lecce's (1971) conclusion that closure in piglets results from changes in the properties of intestinal epithelium in situ rather from a replacement of cells. It is possible that the intestinal epithelial cells of the neonatal pig retain their capacity to uptake macromolecules throughout their lifespan but unlike the rat, they lose their ability to transport macromolecules to the circulation some time before they are shed from the villi.

Various factors have been determined to have an impact on intestinal closure in neonatal piglets.

1. Starvation: piglets feed either porcine or bovine colostrum lost their capacity to uptake and transport macromolecules within 12 to 36 h post-partum while starved piglets retained their capacity for up to 106 h (Lecce et al., 1973).
2. Colostrum and their components: Lecce and Morgan (1962) fed 300 ml of porcine colostrum within 20 h post-birth and found that invoke closure. Werhahn and others (1981) fed 15 g of lactose during the first 24 h and saw a reduction of absorption of IgG by 26% when compared to control while treatment of 54 g of lactose reduced absorption by 94%.

3. Iron: Bokhout and Stok (1980) administered dextran coupled iron to neonatal piglets immediately after birth and prior to nursing and they found a reduction of IgG uptake from colostral milk.
4. Carbohydrates: Broughton and Lecce (1970) found that glucose induced intense pinocytotic activity in the intestinal epithelial cells of the jejunum just like colostrum had. They conducted a study comparing the effects on macromolecular uptake of ^{125}I-PVP by feeding either a 5% glucose/electrolyte solution or a commercial milk replacer for the first 24 to 48 h post-partum and prior to colostrum administration to macromolecular uptake in piglets fed colostrum from birth. They determined that piglets fed colostrum from birth absorbed 50% of their test dose within six h post-partum and there was a rapid increase in serum IgG levels. Piglets that received the glucose/electrolyte solution absorbed 40% of their test dose administered 24 h or 36 h post-partum and had similar serum IgG levels to the colostrum fed piglets. However the piglets fed a milk replacer absorbed only 10% of their test dose and essentially no IgG levels.
5. Endocrine factors: Treatment with various adrenocortical steroids have shown to induce premature cessation of the uptake and transport of macromolecules. Halliday (1959) showed that corticosteroids caused premature intestinal closure. Evidence showed that thyroxine may also influence intestinal development. Payne and Marsh (1962) showed that when starved piglets were injected with cortisone acetate closure occurred 48 to 72 h after. Svendsen et al. (1986) observed that in naturally reared neonatal piglets, closure at 18 h post-partum was associated with an increase in serum

immunoreactive insulin concentration. They speculated that insulin may be involved in closure by initiating the synthesis of membrane structural proteins in the enterocytes.

SYNTHESIS OF GLUTAMINE AND ITS ROLE IN METABOLISM AND PHYSIOLOGY

Glutamine is one of the 20 amino acids encoded by the standard genetic code. Its side chain is an amide formed by replacing the side-chain hydroxyl of glutamic acid with an amine functional group. It can therefore be considered the amide of glutamic acid. Its codons are CAA and CAG. In human blood and pig plasma, glutamine is the most abundant free amino acid with a concentration of about 500-900 μmol/l and 500-600 μmol/l respectively. Herbert (1966) reported that the tissue concentrations of most amino acids are of the same order as the plasma concentration with five exceptions (glutamate, glutamine, aspartate, alanine and glycine) in the case of rat tissues. The tissue concentrations of these five exceptional amino acids can be 10 to 50 times higher than the plasma concentration. Another significant observation is that is the most abundant AA in sow's milk and second most abundant AA in milk proteins (Wu and Knabe, 1994; Haynes et al., 2009). Li et al. (2009b) reported that glutamine is synthesized from branched-chain AA (BCAA) and glucose-derived α-ketoglutarate via BCAA transaminase and glutamine synthetase in lactating porcine mammary tissue (Figure 2).

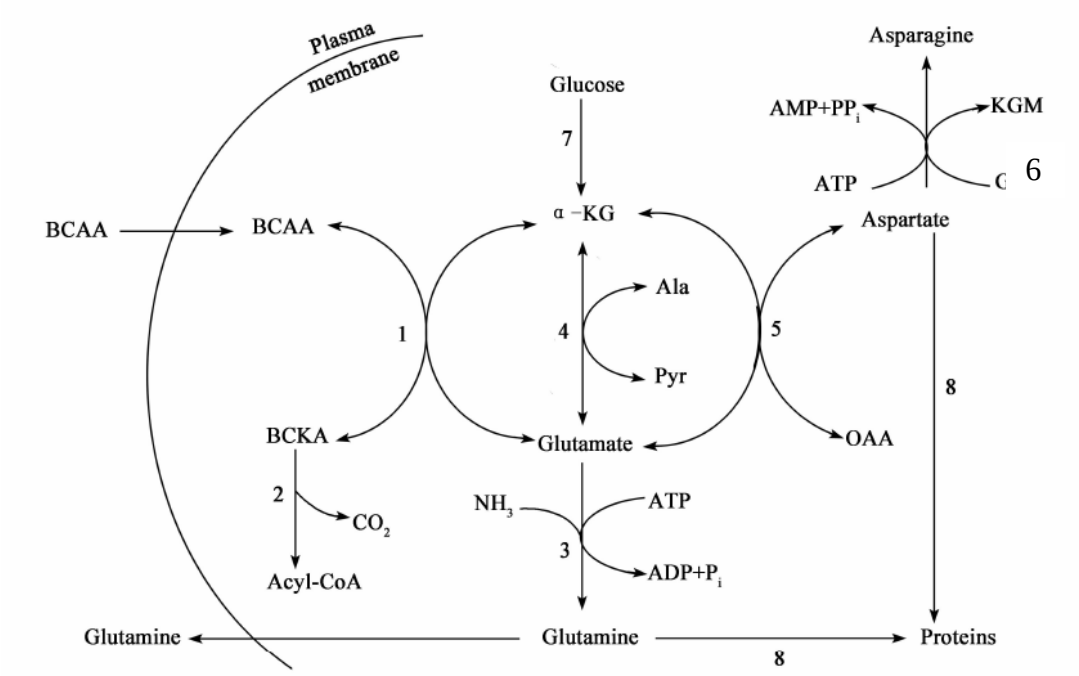


Figure 2. Pathways of glutamine synthesis in pigs. Enzymes that catalyze the indicated reactions are: 1) BCAA transaminase; 2) branched-chain α-ketoacid dehydrogenase; 3) glutamine synthetase; 4) glutamate-oxaloacetate transaminase; 5) glutamate-pyruvate transaminase; 6) asparagine synthetase; 7) glucose metabolism via glycolysis and the Krebs cycle; 8) protein synthesis. (Li et al., 2009b).

Skeletal muscle is the major site of de novo synthesis of glutamine in animals whereas in the intestine of neonatal and adult pigs, the synthesis is negligible (Haynes et al., 2009; Li et al., 2009a). The two major sites of extensive glutamine usage are the enterocytes of the small intestine (Wu et al., 1995) and bacteria present in the lumen of the intestine (Dai et al., 2010). The small intestine is not only responsible for terminal digestion and absorption of nutrients, but it also plays an important role in catabolism of arterial glutamine and dietary amino acids (Wu, 1998). Others research indicate that glutamine is a primary energy source of enterocytes and immune cells (Windmueller and Spaeth, 1980; Newsholme et al., 1999)

providing ATP for intracellular protein turnover, nutrient transport though the plasma membrane, cell growth and migration, as well as the maintenance of cell integrity (Curthoys and Watford, 1995). Results reported by Windmueller and Spaeth (1980) in adult rat small intestine, CO_2, lactate, alanine and glucose account for 56-64, 16-20, 4-8 and 2-10% of the total catabolized carbons of luminal glutamine, glutamate and aspartate, respectively. These results indicate that amino acids, rather than glucose, are the major fuel for the small intestinal mucosa.

The other major known functions of Glutamine are:

1. Precursor for the synthesis of purine and pyrimidine which are essential for intraepithelial lymphocytes, embryonic cells and trophoblasts (Curi et al., 2005).
2. Precursor for the synthesis of arginine (Wu et al., 1995).
3. Increases the expression of genes (ornithine decarboxylase, heat-shock proteins, and nitric oxide synthase) that regulate nutrient metabolism, antioxidative response, and cell survival (Curi et al., 2005; Haynes et al., 2009).
4. Precursor of ornithine though the stimulation of ornithine decarboxylase (ODC), the rate limiting enzyme in polyamine synthesis which is required for intestinal cell proliferation and repair.
5. Enhances the activity of the mammalian target of rapamycin (mTOR), a protein kinase that regulates intracellular protein synthesis.
6. Modulates NF-kappa B (NFkB) signaling pathway by reducing the level of I kappa B-alpha, leading to an increase in NF-kappa B within the nucleus in peritoneal macrophages (Rogero et al., 2010).

7. Precursor for the synthesis of N-acetylglucosamine-6-phosphate, a common substrate for the synthesis of glycoproteins such as mucins that are particularly rich in intestinal mucosal cells and glutathione, the most abundant small-molecular-weight antioxidant in cells (Reeds and Burrin, 2001). (Figure 3).

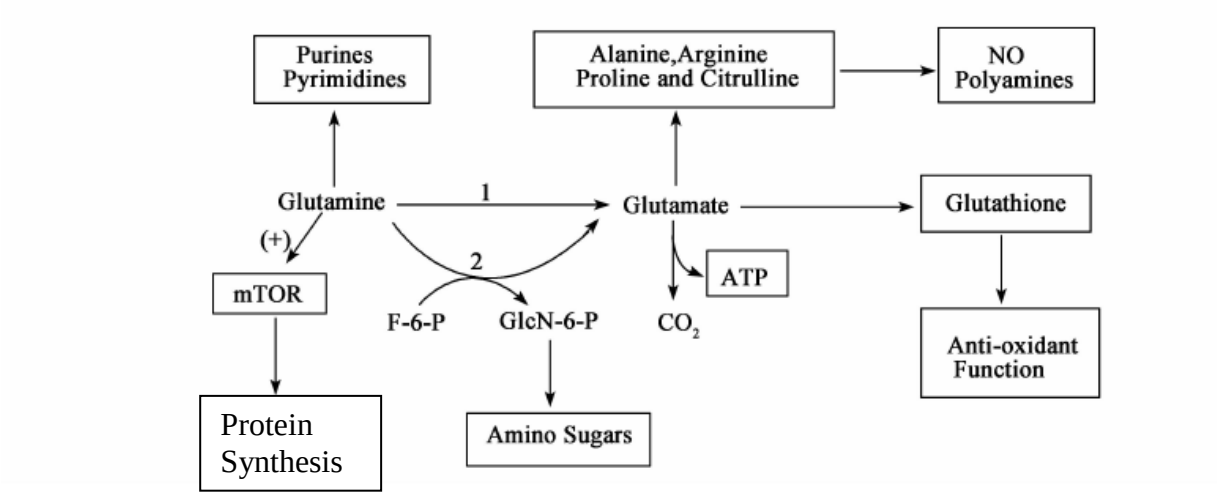


Figure 3. Possible mechanisms responsible for the beneficial effect of glutamine in intestinal barrier function and growth (adapted from Wu, 2007).

Hosam et al. (1991) studied the effect of L-glutamine, the principal intestinal fuel, on proliferation of a porcine jejunal cell line, IPEC-J2. They found that in cells synchronized by serum deprivation for 4 h, glutamine stimulated ornithine decarboxylase (ODC) in a dose- and time-dependent manner, with maximal effects at 10mM in 3 h ($P < 0.01$). Similar effects were seen for the structurally related amino acid L-asparagine and serum. The glutamine effect on ODC was specific, as isosmolar mannitol, glucose, methyl-β-D-glucoside, L-phenylalanine, ammonia, and aminoisobutyric acid were ineffective (Figure 4).

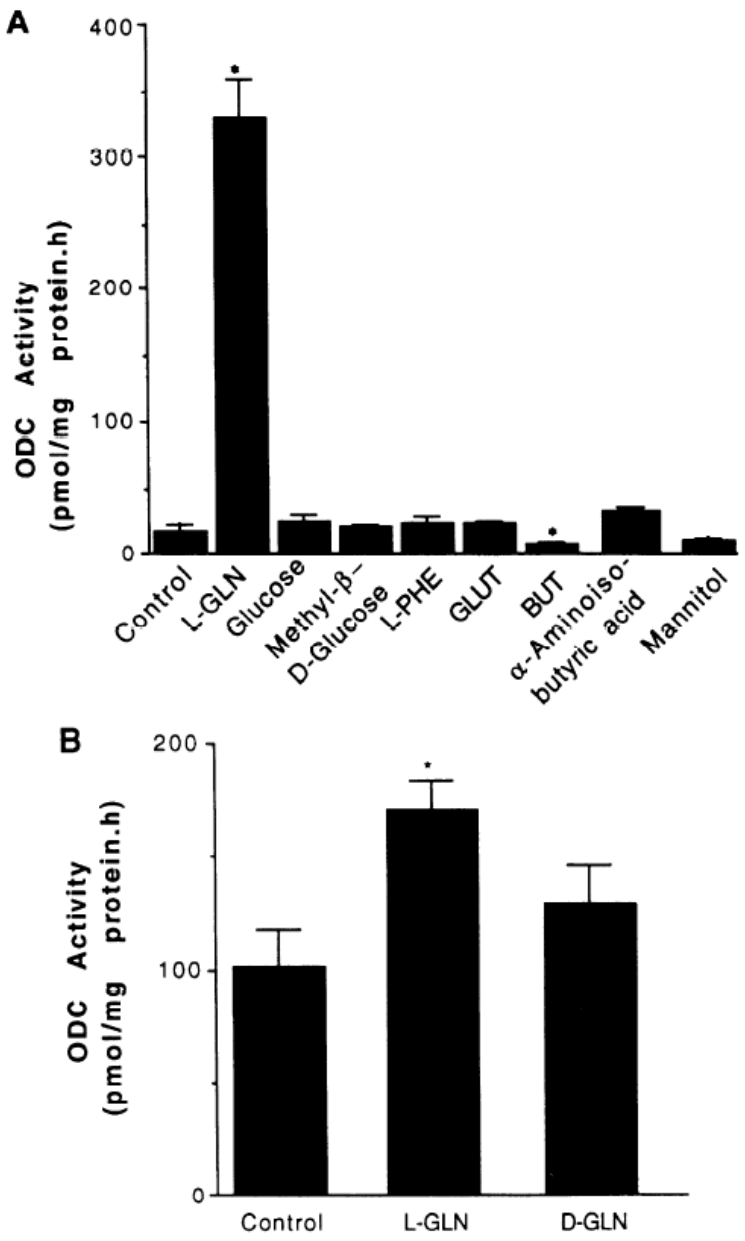


Figure 4. Specificity of Glutamine effect on ODC activity. IPEC-J2 cells were made quiescent by serum starvation for 4 h in EBSS plus 0.1% BSA and then stimulated with different treatments (each at 10mM) for 3 h. *Significantly different from the control, $P < 0.05$. Panel A: n = 3-7 observations from 1 passage of cells. Panel B: n = 10-12 observations from 3 passage of cells. (Hosam et al., 1991).

Rhoads and others (1997) found that ambient glutamine concentration regulates proliferative rate of rat IEC-6 cells whereas additional glutamine provides a mitogenic trigger. They altered glutamine concentration within the physiological range immediately after cells were plated, allowed cells to attach overnight and subsequently measured ^{3}H thymidine incorporation for 24 h. Four levels of glutamine were chosen: 0 mM, 0.7 mM to mimic levels in plasma, 2.5 mM to mimic levels in standard tissue culture media and 3.5 mM to mimic maximum levels in porcine colostrum and levels in the intestinal mucosa. Figure 5 indicates that when the ambient concentration of glutamine was 0.7 mM, steady-state thymidine incorporation was maximal (15-fold the rate in media without glutamine; $P < 0.001$). After the overnight incubation in media with the four different glutamine concentrations, the subsequent addition of 2.5 mM glutamine produced a maximal response in cells starved of glutamine (22 ± 2-fold increases of ^{3}H thymidine incorporation compared with 12 ± 1-fold increases after application of 0.7 mM glutamine, $P < 0.01$). However, in cells continuously bathed in higher concentrations of glutamine (2.5 or 3.5 mM), subsequent additions of 2.5 mM glutamine significantly increased proliferation by another 46 ± 1% (gray bars; $P = 0.02$). These results indicate that serum levels of glutamine are sufficient to sustain a rapid rate of intestinal crypt cell proliferation and an addition of glutamine (concentration of ˜ 2.5 mM) has a triggering effect on proliferation.

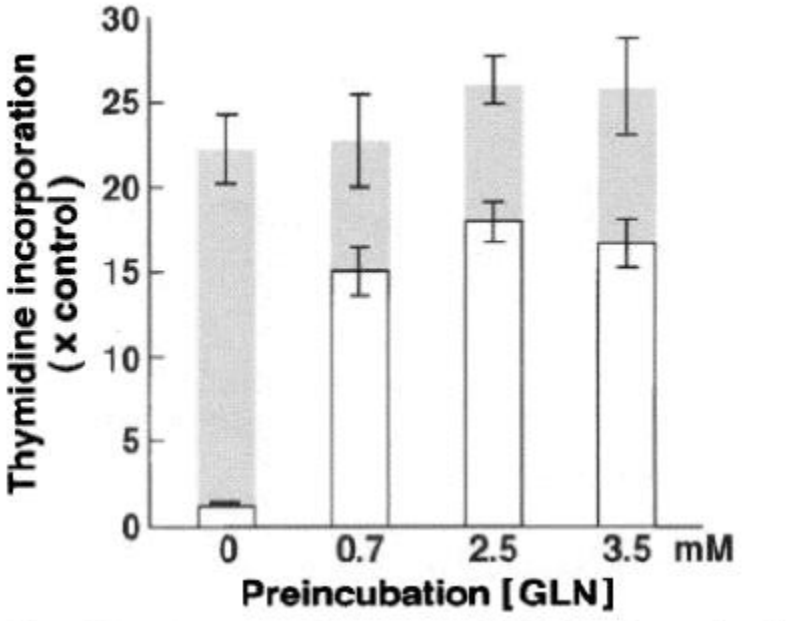


Figure 5. Thymidine incorporation assay of IEC-6 cells preincubated with different concentrations of glutamine. IEC-6 cells were preincubated overnight in glutamine at 0, 0.7, 2.5 or 10 mM. Glutamine (2.5 mM) was added with ^{3}H thymidine 24 h later and radiolabel incorporation was determined. (Rhoads et al., 1997).

Argenzio and others (1994) have demonstrated that some nutrients specially glutamine are capable of driving the Na^{+}/H^{+} exchanger (NHE) even in the presence of villus atrophy associated with viral diseases such as Transmissible Gastroenteritis (TGE) and rotavirus. The NHE family is probably the most important mechanism for transport of Na^{+} into the cells. These transporters (primarily NHE3 and NHE2) are expressed on the apical membrane in the intestinal mucosa and the mucosa of the proximal portion of the colon (Figure 6). TGE is a viral enteric disease of pigs caused by a coronavirus that induces severe villus atrophy and loss of mature absorptive epithelium, leaving a stunned villus lined by a relatively immature

epithelium. TGE-infected porcine intestine has markedly reduced brush-border enzymatic activities and impaired Na^+ and CL^- absorption.

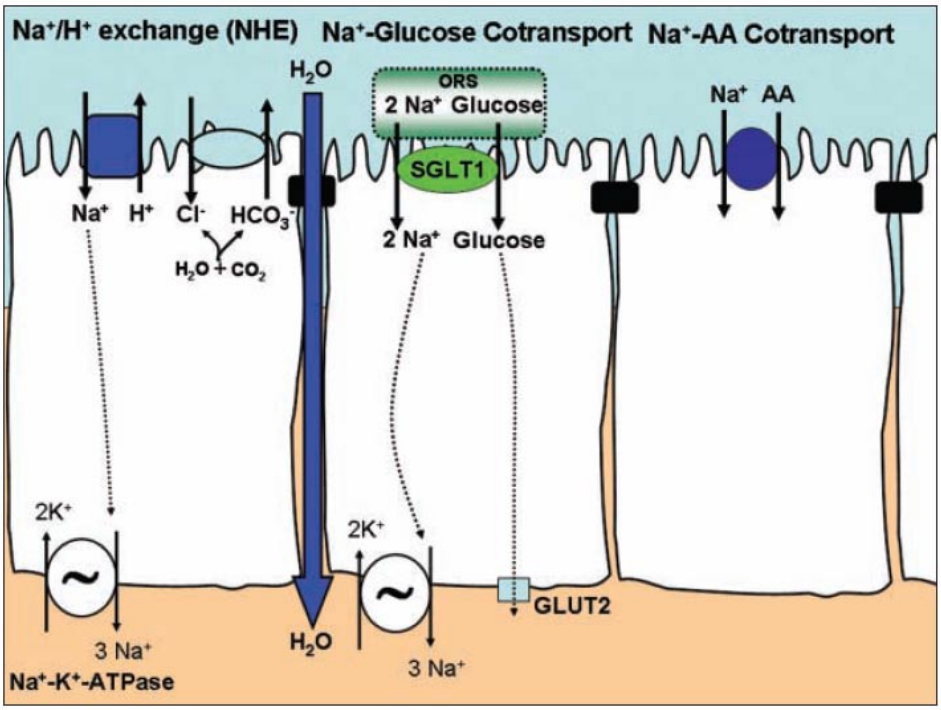
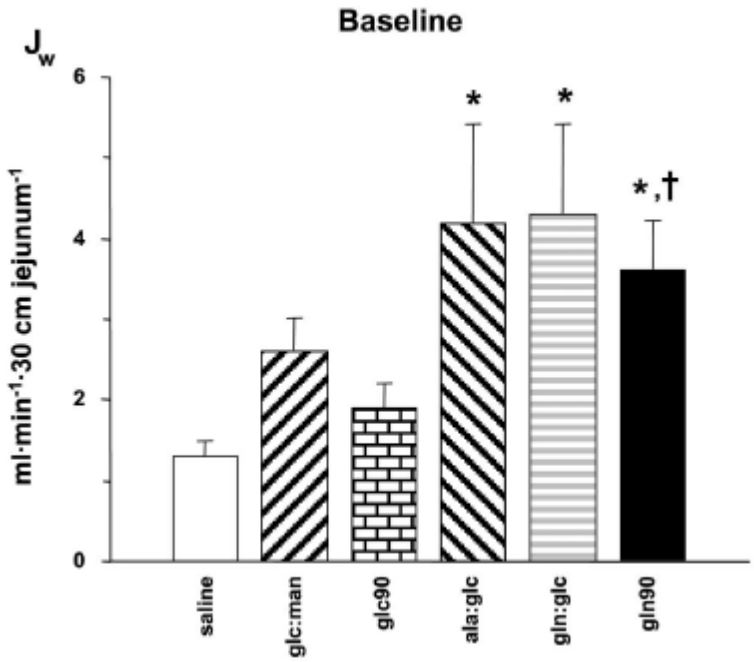


Figure 6. Illustration of the mechanisms of Na^+ absorption across intestinal epithelial cells. GLUT2 = Glucose transporter 2. ORS = Oral Rehydration Solution (Moeser, 2006).

Coeffier et al. (2005) studied the effect of glutamine on jejunal absorption during hypersecretion in six healthy individuals assessed with a triple-lumen tube either at baseline or during PGE_1-induced hypersecretion (0.1 $\mu g \cdot kg^{-1} \cdot min^{-1}$) in random order. Isosmolar solutions containing polyethylene glycol 4000 as nonabsorbable marker were infused in the jejunum at 10 ml/min over a 1-h periods: saline, glucose-mannitol 45:45 mM, glucose 90 mM, alanine-glucose 45:45 mM, glutamine-glucose 45:45 mM and glutamine 90 mM. At baseline, glutamine- and alanine-containing solutions induced a threefold increase of water and sodium

solution ($P < 0.05$). PGE_1-induced hypersecretion was reduced ($P < 0.05$) by solutions of alanine-glucose, glutamine-glucose and glutamine 90 mM and reversed to absorption by alanine-glucose and glutamine-glucose (Figure 7). They concluded that glutamine stimulates water and electrolyte absorption in human jejunum, even during experimental hypersecretion. These results support considering the use of glutamine-containing solutions for the rehydration and the nutritional support of patients with secretory diarrhea.

Figure 7. Water (top) and sodium (bottom) net fluxes in six healthy humans studied during baseline and PGE_1-induced hypersecretion. Negative values denote net secretion into jejunal lumen; positive values denote net absorption from lumen (Coeffier et al., 2005). Gln = Glutamine, Glc = Glucose, Ala = Alanine and Man = Mannitol. Bars represent means ± SE.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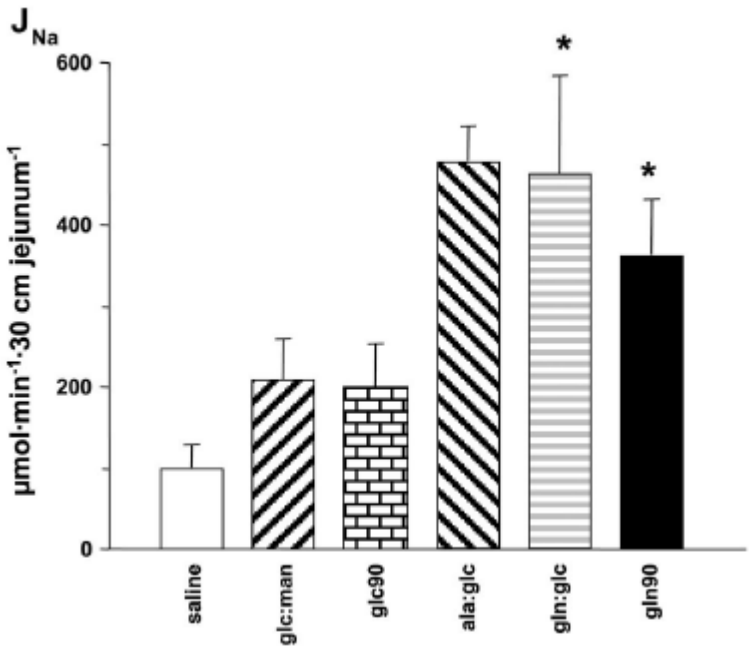
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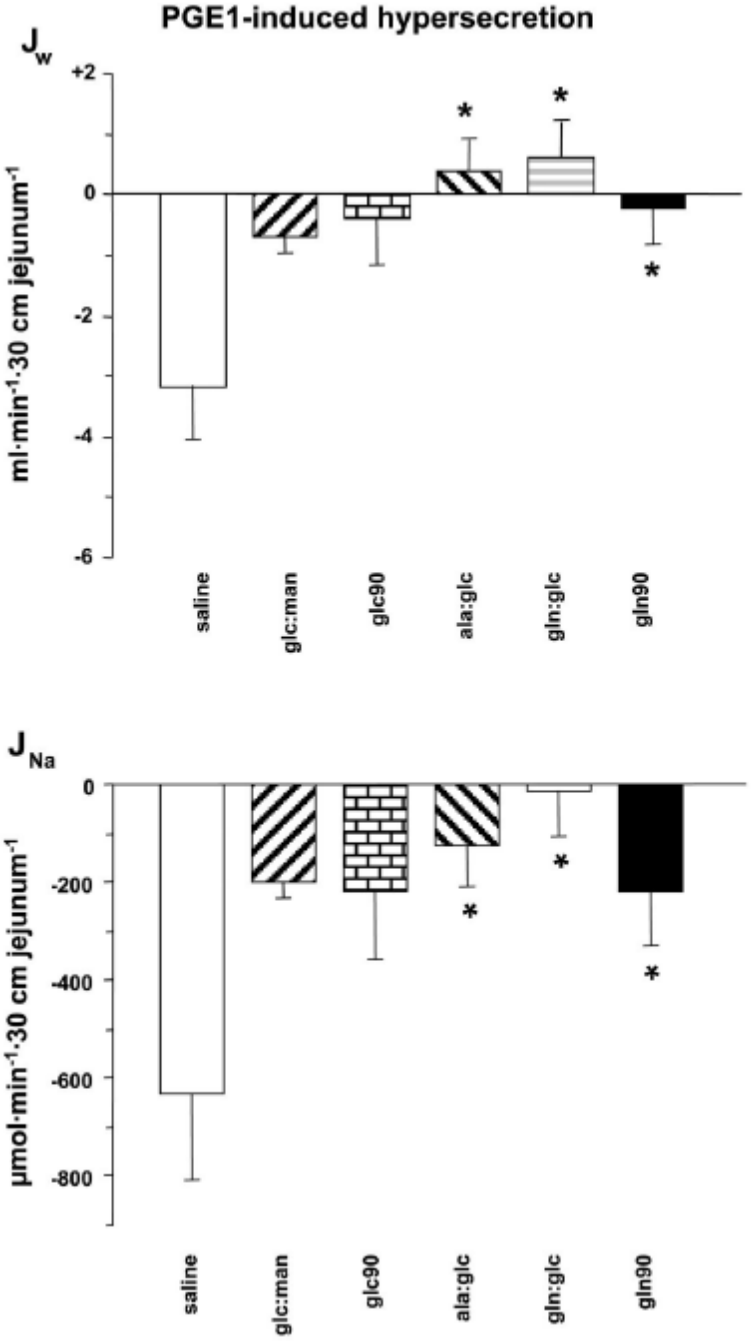
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These results are in agreement with those found by Hoffman and others (2010). They studied the effect of acute L-alanyl-L-glutamine (AG; Sustamine^{TM}) ingestion on performance changes and markers fluid regulation, immune, inflammatory, oxidative stress, and recovery in response to exhaustive endurance exercise, during and in the absence of dehydration. Ten physically active males (20.8± 0.6 yr, 77.4 ± 10.5 kg and 12.3 ± 4.6% body fat) participated in the study. In the first visit (T1), blood was withdrawn and performed maximal exercise as a baseline (BL). In the next 4 trials, they were rehydrated to -2.5% of their baseline body mass. For T2, subjects achieved their goal weight and were not rehydrated. During T3-T5, subjects reached their goal weight and then rehydrated to 1.5% of their baseline body mass by drinking either water (T3) or two different doses (T4 and T5) of the AG supplement (0.05 $g.kg^{-1}$ and 0.2 $g.kg^{-1}$, respectively). They were then asked to exercise until they elicit 75% of their VO_2 max. During T2-T5 blood draws occurred once goal body mass was achieved (DHY), immediately prior to the exercise stress (RHY), and immediately following the exercise protocol (IP). Blood samples were analyzed for glutamine, potassium, sodium, aldosterone, arginine vasopressin (AVP), C-reactive protein (CRP), interleukin-6 (IL-6), malondialdehyde (MDA), testosterone, cortisol, ACHT, growth hormone and creatine kinase. Results showed significantly greater glutamine concentrations for T5 than T2-T4 at RHY and IP (Figure 8). When they examined performance changes (difference between T2-T5 and T-1), significantly greater times to exhaustion occurred during T4 and T5 compared to T2 (Figure 9), they concluded that AG supplementation provided a significant ergogenic effect by increasing time to exhaustion during mild hydration stress and this effect was likely mediated by an enhanced fluid and electrolyte uptake.

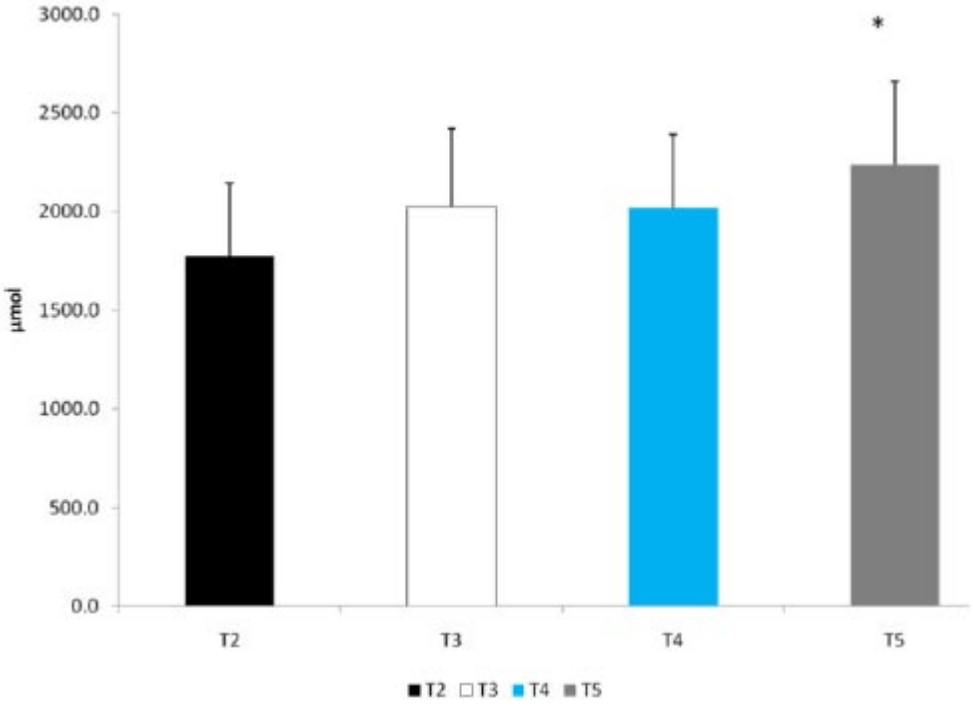

Figure 8. Area under the Curve (AUC) Glutamine concentration. *= significantly different than T2. T2 = not rehydrated, T3 = rehydrated with drinking water, T4 = rehydrated with a solution of L-alanyl-L-glutamine (AG; Sustamine^{TM}) at 0.05 g.kg^{-1} and T5 = rehydrated with a solution of L-alanyl-L-glutamine (AG; Sustamine^{TM}) at 0.2 g.kg^{-1}. (Hoffman et al., 2010).

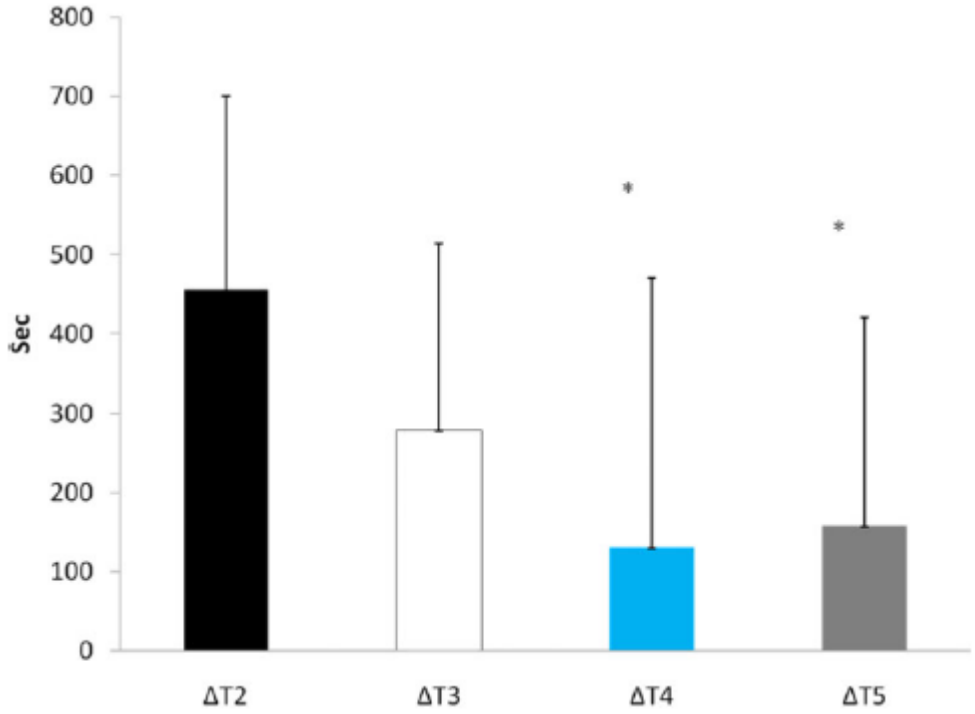

Figure 9. Δ Time to exhaustion. * = significantly different from ΔT2. T2 = not rehydrated, T3 = rehydrated with drinking water, T4 = rehydrated with a solution of L-alanyl-L-glutamine (AG; Sustamine^{TM}) at 0.05 g.kg^{-1} and T5 = rehydrated with a solution of L-alanyl-L-glutamine (AG; Sustamine^{TM}) at 0.2 g.kg^{-1}. (Hoffman et al., 2010).

FORWARD REFLECTIONS ON THESIS RESEARCH WITH PRACTICAL IMPLICATIONS

The study of the impact of lactation length and weaning weight on long-term growth and viability of the progeny without adjusting for the birth weight effect has never been studied and is the subject of thesis chapter 2. It is of extreme importance for breeding stock companies to determine the value of 1 kg of body weight extra at weaning. This will allow them to make an informed decision in terms of how much selection pressure to place on dam milking ability. Many studies (Mahan, 1993; Mahan and Lepine, 1991) have followed progeny from birth until market weight where birth weight was

confounded with subsequent growth performance. Understanding the effect of the time pigs spend with the sow on subsequent health and growth will allow swine professionals to determine the most efficient weaning age of the progeny. This understanding has significant financial implications.

Due to the increased litter size of modern maternal genotypes, it is more crucial than ever to understand the importance of managing sow colostrum and/or colostrum replacement products to maximize piglet's health and consequently survival during the suckling period and beyond (the subject of thesis chapters 3 & 4). Colostrum itself plays primary role in the induction of intestinal closure. The formation of colostrum in the sow begins about one month prepartum with an intensive transfer of immunoglobulins from the serum to the udder (Jonsson, 1973). This process virtually ceases after parturition resulting in a sharp decrease of the IgG concentration in the colostrum (Frenyo et al., 1981; Klobasa et al., 1986). Blecha and co-workers (1998) indicated that the IgG concentration of colostrum is highest during the birth process and decreases during the first day of lactation. These authors also confirmed that IgG absorption by the piglet starts declining 24 h postpartum. Accordingly, if there is an interest in supplementing a colostrum replacement product, it must be provided immediately after birth.

Finally, the vast knowledge of the role of functional AA's such as glutamine and others (arginine, glutamate, proline, leucine, cysteine and tryptophan) provides the scientific basis for nutritionists to revise current nutrient requirements for livestock especially in weaned pigs. This assessment is the subject of thesis chapter 5. Results reported herein

indicate that strong consideration must be given to Gln and glutamate as a nutritionally essential amino acid for post-weaning pig diets.

LITERATURE CITED

1. Allen, W.D. and P. Porter. 1973. The relative distribution of IgM and IgA cells in intestinal mucosa and lymphoid tissues of the young unweaned pig and their significance in ontogenesis of secretory immunity. Immunolology. 24:493.
2. Argenzio, RA., J.M. Rhoads, and M. Armstrong. 1994. Glutamine stimulates prostaglandin-sensitive Na^+-H^+ exchange in experimental porcine cryptosporidiosis. Gastroenterology 106:1418-1428.
3. Auldist, D.E., L. Morrish, P. Eason and R.H King. 1998. The influence of litter size on milk production of sows. J. Anim. Sci. 67:333-337.
4. Auldist, D.E., D. Carlson, L. Morrish, C.M. Wakeford and R.H King. 2000. The influence of suckling interval on milk production. J. Anim. Sci. 678:2026-2031.
5. Averette, L.A., J. Odle, M.H. Monaco and S.D. Donovan. 1999. Dietary fat during pregnancy and lactation increases milk fat and IGF-1 concentrations and improves neonatal growth rates in swine. J. Nutr. 129:2123-2129.
6. Bauman, D.E. and W.B. Curie. 1980. Partitioning of nutrients during pregnancy and lactation: A review mechanisms involving homeostasis and homeorhesis. J. Dairy Sci. 63:1514-1529.
7. Bjorkman, P.J. and P. Parham. 1990. Structure, function and diversity of class I major histocompatability complex molecules. Annu. Rev. Biochem. 90:253-288.

8. Bland, I.M., J.A. Rooke, A.G. Sinclair, V.C. Bland and S.A. Edwards. 2001. Effects of supplementing the maternal diet with vitamins and vaccinating the sow on immunoglobulin G concentrations in piglet plasma. Proc. Nutr. Soc. 60:72A (Abstr.)

9. Blecha, F. 1998. Immunological aspects: comparison with other species. In: Verstegen, M.W.A., Moughan, P.J., Schama, J.W. (eds.), the lactating sow. Wageningen Pers, Wageningen, pp.23-44.

10. Bokhout, B.A and W. Stok. 1980. Does a drug administered by oral route affect the transfer of maternal antibodies in piglets. Tijdsch. Diergeneesk 105:197-191.

11. Borvak, J., J. Richardson, C. Medesan, F. Another, C. Radu, M. Simionescu, V. Gethie and E.S. Ward. 1998. Functional expression of the MHC class-I related receptor, FcRn, in endothelial cells of mice. Int. Immunol. 10:1289-1298.

12. Boyd, R.D., B.D. Moser, A.J. Lewis, E.R. Peo Jr., R.K. Johnson and R.D. Nimmo. 1981. Effects of maternal dietary energy source on glucose homeostasis, liver glycogen and carcass lipid in the neonatal pig. J. Anim. Sci. 53:1316-1324.

13. Broughton, C.W and J.G Lecce. 1970. Electron microscopic studies of the jejunal epithelium from neonatal pigs fed different diets. J. Nutr. 100:445-449.

14. Burmeiser, W.P., L.N. Gastinel, N.E, Simister, M.L. Blum and P.J. Bjorkman. 1994a. The 2.2 Å crystal structure of the MHC-related neonatal Fc receptor. Nature 372:336-343.

15. Burmeiser, W.P., A.H. Huber and P.J. Bjorkman. 1994b. Crystal structure of the complex between the rat neonatal Fc receptor and Fc. Nature 372:372-383.

16. Butler, J.E. 1979. Immunologic aspects of breast feeding, anti-infectious activity of breast milk. In: T.K. Oliver and T.H. Kirschbaum (ed.) Human Lactation. Semin. Perinatol. 3:255.
17. Cabrera, R.A, R.D. Boyd, S. B. Jungst, E. R. Wilson, M.E. Johnston, and J. L. Vignes, and J. Odle. 2010. Impact of lactation length and piglet weaning weight on long-term growth and viability of the progeny. J. Anim. Sci. 88:2265-2276.
18. Chiston, R., G.Saminadin, H. Lionet and B. Bacon. 1999. Dietary fat and climate alter food intake, performance of lactating sows and their litters and fatty acid composition of milk. J. Anim. Sci. 69:353-365.
19. Coeffier, M., B. Hecketsweiler, P. Hecketsweiler, and P. Dechelotte. 2005. Effect of glutamine on water and sodium absorption in human jejunum at baseline and during PGE_1-induced secretion. J. Appl. Physiolo. 98:2163-2168.
20. Coffey, M.T., R.W. Seerley, R.J. Martin and J.W. Mabry. 1982. Effect of level, source, and duration of feeding of supplemental energy in sow diets on metabolic and hormonal traits related to energy utilization in the baby pig. J. Anim. Sci. 55:329-336.
21. Curi, R., C.J. Lagranha, S.W. Doi, D.F. Sellitti, J. Procopio, T.C. Curi, M. Corless and P. Newsholme. 2005. Molecular mechanisms of glutamine action. J. Cell Physiol. 204:392-401.
22. Curthoys, N.P. and M. Watford. 1995. Regulation of glutamine activity and glutamine metabolism. Annu. Rev. Nutr. 15:133-159.
23. Curtis, J. and F.J. Bourne. 1973. Half lives of immunoglobulins IgG, IgA and IgM in the serum of newborn pigs. Immunolology 24:147.

24. Dai, Z.L., J. Zhang, G. Wu and W.Y. Zhu. 2010. Utilization of amino acids by bacteria from the small pig intestine. Amino acids. Doi: 10 1007 / s00726-010-0556-9.

25. Devillers, N., C. Farmer, J. Le Dividich and A. Prunier. 2007. Variability of colostrum yield and colostrum intake in pigs. Animal 1:1033-1041.

26. Farmer, C and H. Quesnel. 2009. Nutritional, hormonal and environmental effects on colostrum in sows. J. Anim. Sci. 87:56-65.

27. Farmer, C and M.T. Sorensen. 2001. Factors affecting mammary development in gilts. Livest. Prod. Sci. 70:141-148.

28. Frenyo, V., G. Pethes, T. Antal and I. Szabo. 1981. Changes in colostral and serum IgG content in swine in relation to time. Veterinary Research Communications 4:275-282.

29. Fridman, W.H. 1991. Fc receptors and immunoglobulins binding factors. FASEB J. 5:2684-2690.

30. Fridman, W.H. (ed). 1989. Structures and functions of low affinity Fc receptors. Chem. Immunol. 47:1-258.

31. Gaskins, H.R. 1998. Immunological development and mucosal defense in pig intestine. Pages 81-102 in Progress in pig science. J. Wiseman, M.A. Varley and J.P. Chadwick, ed. Notingham Univ. Press, Notingham, UK.

32. Gastinel, L.N., N.E. Simister and P.J. Bjorkman. 1992. Expression and crystallization of a soluble and functional form of an Fc receptor related to class I histocompatability molecules. Proc. Natl. Acad. Sci. 89:638-642.

33. Halliday, R. 1959. The effect of steroid hormones on the absorption of antibody in the young rat. J. Endocrinology 18:56-66.

34. Hardy, R.N. 1965. Intestinal absorption of macromolecules in the newborn pig. *J.* Physiol. 176:19.

35. Harrell, R.J., M.J. Thomas, and R.D. Boyd. 1993. Limitations of the sow milk yield on baby pig growth. Pages 156-164 in Proc. 1993 Cornell Nutr. Conf. Feed Manufacturers. Cornell University, Ithaca, N.Y.

36. Hartmann, P.E., I. McCauley, A.D. Gooneratne and J.L. Whitley. 1984. Inadequacies of sow lactation: Survival of the fittest. In: M. Peaker, R.G. Vernon and C.H. Knight (Ed.) Physiological strategies during lactation. Pp. 301-326. Academic Press, London, UK.

37. Heo, S., Y.X. Xang, Z. Jin, M.S. Park, B.K. Yang and B.J. Chae. 2008. Effects of dietary energy and lysine intake during late gestation and lactation on blood metabolites, hormones, milk composition and reproductive performance in primiparous sows. Can. J. Anim. Sci. 88:247-255.

38. Hoffman, J.R., N. A. Ratames, J. Kang, S.L. Rashti, N. Kelly and A. Gonzalez. 2010. Journal of the international society of sports nutrition 7:8.

39. Hosam, M.K., R.A. Argenzio, W. Chen, H.M. Berschneider, A.D. Stiles, J.K. Westwick, R.A. Rippe, D.A. Brenner and J.M. Rhoads. 1995. L-Glutamine and L-Asparagine stimulate ODC activity and proliferation in a porcine jejunal enterocytes line. The American Physiological Society: G591-599.

40. Huber, A.H., R.F. Kelley, L.N. Gastinel and P.J. Bjorkman. 1993. Crystallization and stoichiometry of binding a complex between a rat intestinal Fc receptor and Fc. J. Mol. Biol. 230:1077-1083.

41. Jackson, J.R., W.L. Hurley and R.A. Easter, A.H. Hansen and J. Odle. 1995. Effects of induced or delayed parturition and supplemental dietary fat on colostrum and milk composition. J. Anim. Sci. 73:1906-1913.

42. Jensen, A.R., J. Elnif, D.G. Burrin and P.T. Sangild. 2001. Development of intestinal immunoglobulin absorption and enzyme activities in neonatal pigs is diet dependent. J. Ntr. 131 (12): 3259-3265.

43. Jensen, P., G. Sangel and B. Algers. 1991. Nursing and sucking behavior of semi-naturally kept pigs during the first 10 days of post-partum. Appl. Anim. Behav. Sci. 31:195-203.

44. Jones, E.A. and T.A. Waldman. 1972. The mechanism of intestinal uptake and transcellular transport of IgG in the neonatal rat. J. Clin. Invest. 51:2916-2927.

45. Jonsson, A. 1973. Transfer of immunoglobulins from mother to offspring in the pig. Acta vet. Scand. Suppl. 43:1-64.

46. Kerbert, J.D., R.A. Coulson and T. Fernandez. 1966. Free amino acids in caiman and rat. Comp. Biochemi. 17:583-598.

47. Kim, J.K, M.F. Tsen, V. Gethie and E.S. Ward. 1994. Localization of the site of the murine IgG1 molecule that is involved in binding to the murine intestinal Fc receptor. Eur. J. Immunol. 24:2429-2434.

48. King, R.H., B.P. Mullan, F.R. Dunshea and H. Dove. 1997. The influence of piglet body weight on milk production of sows. Livest. Prod. Sci. 47:169-174.

49. Klobasa, F. J.E. Butler, E. Werhahn and F. Habe. 1986. Maternal-neonatal immunoregulation in swine: Influence of multiparity on the novo immunoglobulin synthesis by piglets. Vet. Immunol. Immunopathol. 11:149-159.

50. Klobasa, F., E. Werhahn and J.E. Butler. 1987. Composition of sow milk during lactation. J. Anim. Sci. 64:1458-1466.

51. Klobasa, F., J.E. Butler, E. Werhahn and F. Habe. 1986. Maternal-neonatal immunoregulation in swine.II. Influence of multiparity on de novo synthesis by piglets. Vet. Immunol. Immunopath. 11:149.

52. Klobasa, F., F. Habe, E. Werhahn and J.E. Butler. 1981. Regulation of humoral immunity in the piglet by immunoglobulin of maternal origin. Res. In Vet. Sci. 31:195-206.

53. Korhonen, H., P. Marnila and H.S. Gill. 2000. Bovine milk antibodies for health. British Journal of Nutrition 84: S135-S146.

54. Lecce, J.G and C.W. Broughton. 1973. Cessation of uptake of macromolecules by neonatal guinea pig, hamster and rabbit intestinal epithelium (closure) and transport to the blood. J. Nutrition 103:744-750.

55. Lecce, J.G and D.O. Morgan. 1962. Effect of dietary regimen on cessation of intestinal absorption of large molecules (closure) in the neonatal pig and lamb. J. Nutr. 70:263.

56. Lewis, A.J., V.C. Speer and D.G. Haught. 1978. Relationship between yield and composition of sow's milk and weight gains of nursing pigs. J. Anim. Sci. 47:634-638.

57. Li, P., D.A. Knabe, S.W. Kim, C.J. Lynch, S.M. Hutson and G. Wu. 2009b. Lactating porcine mammary tissue catabolizes branched-chain amino acids for glutamine and aspartate synthesis. J. Nutr. 139:1502-1509.

58. Li, X.L., F.W. Bazer, H. Gao, W. Jobgen, G.A. Johnson, P. Li, J.R. McKnight, M.C. Satterfield, T.E. Spencer and G. Wu. 2009a. Amino acids and gaseous signaling. Amino acids 37:65-78.

59. Mackenzie, N.M. and K.D. Keeler. 1984. Isotype specificity in the binding of mouse immunoglobulins to brush borders isolated from the neonatal mouse small intestine. Biochem. Soc. Trans. 12:747.

60. Mahan, D.C. 1993. Effect of weight, split-weaning, and nursery feeding programs on performance responses of pigs to 105 kilograms body weight and subsequent effects on sow rebreeding interval. J. Anim. Sci. 71:1991-1995.

61. Mahan, D.C., and A.J. Lepine. 1991. Effect of weaning weight and associated nursery feeding programs on subsequent performance to 105 kilograms of body weight. J. Anim. Sci. 69: 1370-1378.

62. Mateo, C.D., D.N. Peters and H.H Stein. 2004. Nucleotides in sow colostrum and milk at different stages of lactation. J. Anim. Sci. 82:1339-1342.

63. McCarthy, K.M., Y. Yong and N.E. Simister. 2000. Bidirectional transcytosis of IgG by the rat neonatal Fc receptor expressed in a rat kidney cell line: a system to study protein transport across epithelia. Journal of Cell Science 113:1277-1285.

64. Moeser, A. 2006. "Mechanisms of Prostaglandin-Stimulated Recovery of Mucosal Barrier Function in the Ischemia-Injured Porcine Intestine: Role of Intestinal Ion

Transport". PhD Dissertation. Department of Population Health and Pathobiology. College of Veterinary Medicine. North Carolina State University, Raleigh, NC 27695.

65. Moon, H.W. 1971. Epithelial cell migration in the alimentary mucosa of the suckling pig. Proceedings of the society of experimental biology and medicine137:151-154.
66. Mostov, K.E., M. Friedlander, G. Blodel. 1984. The receptor for transepithelial transport of IgA and IgM contains multiple immunoglobulin-like domains. Nature 308:37-43.
67. Mostov, K.E. and N.E. Simister. 1985. Transcytosis. Cell 43;389-390.
68. Newsholme, P., R. Curi, T.C. Pithon Curi, C.J. Murphy, C. Garcia and M. Pires de Melo. 1999. Glutamine metabolism by lymphocytes, macrophages and neutrophils: its importance in health and disease. J. Nutr. Biochem. 10:316-324.
69. Newsholme, P. 2001. Why is L-glutamine metabolism important to cells of the immune system in health, post injury, surgery or infection? J. Nutr. 131:2515S-2522S.
70. Odle, J., R.T. Zijlstra and S.M. Donovan. 1996. Intestinal effects of milkborne growth factors in neonate of agricultural importance. J. Anim. Sci. 74:2509-2522.
71. Payne, L.C and C.L. Marsh. 1962. Gamma globulin absorption in the baby pig: The non-selective absorption of heterologous globulins and factors influencing absorption time. J. Nutr. 76:151.
72. Pinelli-Saavedra, A., A.M. Calderon de la Barca, J. Hernandez, R. Valenzuela and J.R. Scaife. 2008. Effects of supplementing sows fed with α-tocopherol acetate and vitamin C on transfer of α-tocopherol to piglet tissues, colostrum and milk: Aspects of immune status of piglets. Res. Vet. Sci. 85:92-100.

73. Popov, S., J.G. Hubbard, J.K. Kim, B. Ober, V. Gethie and E.S. Ward. 1996. The stoichiometry and affinity of murine Fc fragments with the MHC class I-related receptor, FcRn. Mol. Immunol. In Press.

74. Porter, P. 1969. Transfer of immunoglobulins IgG, IgA and IgM to lacteal secretions in the parturient sow and their absorption by the neonatal pig. Biochim. Byophys. Acta. 181:381.

75. Raghavan, M., V.R. Bonagura, S.L. Morrison and P.J. Bjorkman. 1995a. Analysis of the pH dependence of the neonatal Fc receptor/immunoglobulin G interaction using antibody and receptor variants. Biochemistry 34:14649-14657.

76. Raghavan, M., Y. Wang and P.J. Bjorkman. 1995b. Effects of receptor dimerization on the interaction between the class I MHC related Fc receptor and immunoglobulin G. *G.* Proc. Natl.Acad. Sci. 92:11200-11204.

77. Raghavan, M. and P.J. Bjorkman. 1996. Fc Receptors and their interactions with immunoglobulins. Annu. Rev. Cell Dev. Biol. 12:181-220.

78. Redman, D.R. 1979. Prenatal influence of immunocompetence of the neonate. J. Anim. Sci. 49:258-267.

79. Reeds, P.J. and J. Odle**.** 1996. Pigs as models for nutrient functional interaction. In: Advances in Swine in Biomedical Research (M. Tumbleson and L. Schook, eds.) Plenum Press, New York.

80. Reeds, P.J. and D.G. Burrin. 2001. Glutamine and the bowel. J. Nutr. 131:2505-2508.

81. Rhoads, J.M., A.D. Cox, R.A. Argenzio, W. Chen, H.M. Berschneider, J.K. Westwick, R.A. Rippe, and D.A. Brenner. 1997. L-Glutamine stimulates intestinal cell

proliferation and activates mitogen-activated protein kinases. The American Physiological Society: G943-953.

82. Rodewald, R. and J.P. Kraehenbuhl. 1984. Receptor mediated transport of IgG. J. Cell. Biol. 99:159-164.
83. Rogero, M.M., P. Borelli and R.A. Fock. 2010. Effects of glutamine on the Nuclear Factor-kappa B (NFkB) signaling pathway of murine peritoneal macrophages. Amino acids 39(2): 435-441.
84. Sangild, P.T., J.f. Trahair, M.K. Loftager and A.L. Fowden. 1999. Intestinal macromolecular absorption in the fetal pig infusion of colostrum in utero. Pediatr Res. 45: 595-602.
85. Sangild, P.T. 2003. Uptake of colostral immunoglobulins by the compromised newborn farm animals. Acta Vet Scan Suppl 98:105-122.
86. Simister, N.E. and A.R Rees. 1985. Isolation and characterization of an Fc receptor from neonatal rat small intestine. Eur. J. Immunol. 15:733-738.
87. Simister, N.E. and K.E. Mostov. 1989. An Fc receptor structurally related to MHC class I antigens. Nature 337:184-187.
88. Simister, N.E. and K.E. Mostov. 1989a. Cloning and expression of the neonatal rat intestinal Fc receptor, a major histocompatability complex class I antigen homolog. Quant. Biol. LIV: 571-580.
89. Souba, W.W., K. Herskowitz, R.M. Salloum, M.K. Chena and T.R. Augsten. 1990. Gut glutamine metabolism. J. Parenter. Enter. Nutr. 14 (Suppl. 4):45S-50S.

90. Speer, C.V., H. Brown, L. Quinn and D.V. Catron. 1959. The cessation of antibody in the young pig. J. Immunol. 83:632.

91. Spencer, J.D., R.D. Boyd, R. Cabrera and G.L. Allee. 2003. Early weaning to reduce tissue mobilization in lactating sows and milk supplementation to enhance pig weaning weight during extreme heat stress. J. Anim. Sci. 81:2041-2052.

92. Stormont, C. 1972. The role of maternal effects in animal breeding: I. Passive immunity in newborn animals. J. Anim. Sci. 35:1275-1279.

93. Svendsen, J., E. Ewert and M.R Wilson. 1971. Immunity to *Escherichia coli* in pigs: the protein levels in secretions from individual mammary glands of sows during the first seven days of lactation. Vet. Sci. 12:448.

94. Svendsen, L.S., B.R. Westrom, J. Svendsen, B.G. Ohlsson, R. Ekman and B.W. Karlsson. 1986. Insulin involvement in intestinal macromolecular transmission and closure in neonatal pigs. J. Ped. Gastr. Nutr. 5:299-304.

95. Townsend, A. and H. Bodmer. 1989. Antigen recognition class I-restricted T lymphocytes. Annu. Rev. Immunol. 7:601-624.

96. USDA. 2007. National Swine Survey: Morbidity/mortality and health management of swine in the U.S. National Animal Health Monitoring System, Animal and Plant Health Inspection Service. Veterinary Services. Washington, DC.

97. USDA. 1993. Transfer of maternal immunity to calves. Unites States Department of Agriculture. Animal and Plant Health Inspection Service. Veterinary Services. Washington, DC.

98. Van de Perre, P. 2003. Transfer of antibody via mother's milk. Vaccine 21:3374-3376.

99. Wallace, K.H and A.R. Rees. 1980. Studies on the immunoglobulin G Fc fragment receptor from neonatal rat small intestine. Biochem. J. 188:9-16.

100. Werhahn, E., F. Klobasa and J. E. Butler. 1981. Investigations of some factors which influence the absorption of IgG by the neonatal piglet. Vet. Immunol. Immunopath. 2:35.

101. Williams, A.F. and A.N Barclay. 1988. The immunoglobulin super family-domain for cell surface recognition. Annu. Rev. Immunol. 6:381-405.

102. Wilson, M.R. 1974. Immunologic development of the neonatal pig. J. Anim. Sci. 38:1018-1021.

103. Windmueller, H.G., and A.E. Spaeth. 1980. Respiratory fuels and nitrogen metabolism in vivo in small intestine of fed rats. Quantitative importance of glutamine, glutamate and aspartate. J. Biol. Chem. 10:107-112.

104. Wu, G and D.A. Knabe, 1994. Free and protein-bound amino acid in sow's colostrums and milk. J. Nutr. 124:415-424.

105. Wu, G., D.A. Knabe, W. Yan and N.E, Flynn. 1995. Glutamine and glucose metabolism in enterocytes of the neonatal pig. Am. J. Physiol. 268:R334-R342.

106. Wu, G. 1998. Intestinal mucosal amino acid metabolism. J. Nutr. 128:1249-1252.

107. Xu, R.J., P.T. Sangild, Y.Q. Zhang and S.H. Zhang. 2002. Bioactive compounds in porcine colostrum and milk and their effects on intestinal development in neonatal pigs. Pages 169-192 in Biology of the intestine in Growing Animals, R. Zabielski, P.C. Gregory and B. Westrom, ed. Elsevier, Amsterdam, The Netherlands.

108. Yang, Y., S. Heo, Z. Jin, J. Yun, P. Shinde, J. Choi, B. Yan and B. Chae. 2008. Effects of dietary energy and lysine intake during late gestation and lactation on blood metabolites, hormones, milk composition and reproductive performance in multiparous sows. Arch. Anim. Nutr. 62:10-21.

109. Zijlstra, R.T., K.Y. Whang, R.A. Easter and J. Odle. 1996. Effect of feeding a milk replacer to early-weaned pigs on growth, body composition, and small intestine morphology compared to suckled littermates. J. Anim. Sci. 74:2948-2959.

CHAPTER 2

IMPACT OF LACTATION LENGTH AND PIGLET WEANING WEIGHT ON LONG-TERM GROWTH AND VIABILITY OF THE PROGENY

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ABSTRACT

A total of 1,034 pigs produced by breeding PIC sows to 2 different PIC terminal sires were used to create 3 distinct weaning weight populations so that post-weaning growth to 125 kg could be studied. The rearing strategies resulted in weights that ranged from 4.1 kg to 11.5 kg by 20 d of age. Sows and corresponding litters were allocated to three treatments: Sow reared (**SR**) (N = 367) for 20 d, sow reared for 14 d (**14W**) (N = 330) and sow reared for 2 d (**2W**) (N = 337). Sows were removed from **2W** and **14W** groups but progeny remained in the crates and received milk replacer ad libitum (for 18 and 6 d, respectively) until the contemporary **SR pigs** were weaned at 20 d of age. The **SR** pigs (6.49 ± 0.15 kg) weighed 1.01 kg less than **14W** pigs (7.5 ± 0.14 kg) and 2.26 kg less than **2W** pigs (8.75 ± 0.14 kg) ($P < 0.05$). The **14W** pigs weighed 1.25 kg less than **2W** pigs ($P < 0.05$). Nursery ADG for the **2W** group (547 g/d) was 35 g/d less than **14W** pigs ($P < 0.05$). **14W** pigs (165 d) required 3 fewer days to reach 125 kg body weight compared to **SR** pigs ($P < 0.05$). The **SR** and **14W** pigs gained 24 and 20 g/d faster in the post-nursery period when compared to **2W** pigs ($P < 0.05$). The **SR** and **2W** pigs consumed 0.10 and 0.12 kg/d less during this period when compared to **14W** pigs (2.32 kg/d) ($P < 0.05$). Gain: feed of **SR** was significantly ($P < 0.05$) improved when compared to the **14W** and **2 W** pigs over a 167-d of age (0.44 vs. 0.42 and 0.42 respectively). Lean percentage was .7 % higher in carcasses from **SR** pigs (55.0 %) compared to carcasses from **2W** pigs (54.3%) when adjusted to a constant hot carcass weight weight ($P < 0.05$). A study of the effect of weaning weight on days to 125 kg was limited to **SR** and **14W** groups since maternal deprivation compromised the **2W** group post-weaning growth. Six weaning weight groups were defined using a normal distribution: 4.6 kg; 5.5 kg;

6.4 kg; 7.3 kg; 8.2 kg; and 9.5 kg. Pigs weighing 5.5 kg at 20-d of age were able to reach 125 kg 8 d sooner (168.8 d) than those weighing 4.6 kg (176.8 d). There was a linear relationship ($P < 0.05$) between weaning weight and ADG in the post-nursery phase of growth. These results suggest that (1) a weaning weight of less than 5.0 kg weaning weight imposes the greatest marginal loss in production output for a 20-d weaning, and that (2) lactation length influences long-term growth, composition of growth and viability of progeny.

INTRODUCTION

Weaning weight is an important factor influencing post-weaning growth. Pigs with heavier weaning weights grow more rapidly post-weaning. Mahan and Lepine (1991) showed pigs weighing 6.8 to 8.2 kg at weaning reached 105 kg approximately 10-d earlier than pigs weighing 4.1 to 5.5 kg. This finding is consistent with Azain (1997), who observed pigs weighing on average of 5.65 kg reached 104 kg of bodyweight 7-d sooner than those weighing 4.5 kg. However it is not clear to us how weaning weight affected subsequent and viability performance when the confounding influence of birth weight was removed.

The immunity acquired by the piglets during the time they spend with the sow is critical for the protection of the piglets against diseases after weaning (Morrow, 2004). The influence of maternal age on the immune competence of her progeny is known to be important but has been more extensively studied in dairy cattle than pigs. Blecha et al. (1983) showed in vivo and in vitro cellular immune responses were compromised in early-weaned piglets. Our study examines how the piglets' long-term growth and viability beyond the colostrum period are influenced by the amount of time they spend nursing their mother.

The objectives of this experiment were (1) to determine the relationship between weaning weight and growth to 125 kg; and (2) to determine the impact of time spent nursing the sow on long-term growth and viability of her progeny. The confounding effect of birth weight was removed by our procedure.

MATERIALS AND METHODS

This study was conducted at the PIC USA research farm located in Gold City, KY. The distance separating the sow farm (site I) from nursery and finish facilities is no more than 500 m. Nursery and finish facilities are on the same site (Site II). The farm's (farrowing, nursery and finish) health status was defined as PRRS negative, Mycoplasma positive and PRV and Brucellosis negative based on serological sampling by the veterinary team in charge of health.

All protocols were under the supervision of licensed veterinarians. Standard operating procedures for animal care were in accord with published guidelines for animal care (FASS, 1999). The experimental animals were not subjected to prolonged constraint or surgical procedures and were humanely treated throughout the experiment.

A total of 112 litters produced by breeding PIC sows to terminal sires were assigned to 3 treatment groups involving different nursing lengths with their sow. The groups remained in their respective crates for 20 d (20 ± 0.2) but the time that the sow remained with the litter was varied: sow reared from birth to weaning (**SR**), 14 d with litter (**14W**), and 2 d with litter (**2W**). The litters of pigs assigned to the **2W** and **14W** groups remained as intact litters and in their crates after the sow was removed. Sow feces was not removed. Piglets in the **2W** and **14W** groups were fed an acidified medicated milk replacer (MR) for 18-d and 6-d

respectively. The MR (Advanced Birthright Nutrition, Delano, MN and Ralco Nutrition, Inc.; Marshall, MN) was medicated with 55 mg/kg of oxytetracycline and 110 mg/kg of neomycin. It was formulated to contain 24.1% crude protein and 18.1% crude fat (Table 1) and was based on dairy milk products and purified soya. The MR was delivered using the Supp-Le-Mate C.S. semi-automated milking system (Soppe Systems, Inc.; Manchester, IA). Fresh milk was mixed daily and chlorinated with calcium hypochlorite pellets (Better Water Industry, Inc.; Tyler, MN) at a ratio of 3.75 g Cl/L, added at a central reservoir and then circulated in a pressurized line (20 to 25 psi) to a series of pig-activated drinkers using a pneumatic pump.

A total of 698 pigs (out of 1,034 pigs) met our weaning weight criteria for the 3 different rearing strategies. Eleven to 12 litters farrowed each week. Each litter was randomly assigned prior to birth within parity to 1 of the 3 groups. Sows were fed lactation diets that exceeded NRC (1998) nutrient specifications assuming pig litter size of 11 and litter growth rate of 2,550 g/d.

Litters assigned to the **SR** control group remained with their sow until the piglets were weaned. Average age at weaning for this group was 19.5 ± 0.2 d of age. Supplemental MR was not provided to SR litters. For this group, the objective was to produce pigs that weighed between 4.1 and 6.6 kg at weaning. Litters assigned to the **14W** group were weaned at 14 d of age by removing the sow. Progeny were then fed an acidified medicated MR ad libitum for 6 d until their contemporary **SR** litters were weaned. The objective of this group was to produce pigs that weighed between 6.8 to 8.4 kg at weaning. Litters assigned to **2W** group were weaned 2-d after birth and then were fed the acidified medicated MR for 18 d. The objective

of this group was to produce pigs that grew without maternal constraint and weighed more than 8.6 kg at 20 d of age.

Piglets were processed within 24 h of birth. Each pig was individually weighed using a digital balance (model 4, Mosdal Scale Systems, Inc.; Broadview, MT) and identified with numbered ear tags in the left and right ears for individual identification. Each pig was given 1 cc of a mixture of iron dextran and penicillin, and 1 cc of Garacin (Gentamicin Sulfate, Schering-Plough Animal Health Corporation, Whitehouse Station, NJ). All pigs received creep feed during the last week of the lactation period to acclimate them to dry food. At 10 d of age, all pigs were vaccinated intramuscularly with 2 mL intramuscularly of Suvaxyn Respifend HPS (Haemophilus Parasuis Bacterin, Fort Dodge, IA. A second vaccination of HPS was given 2 wk later, after relocation to the nursery.

Pigs from the 3 groups were individually weighed at weaning (20 ± 0.2 d of age) using the Mosdal scale. Each pig was tagged in the left ear with a red (**SR**), green (**2W**) or yellow (**14W**) button tag to designate the group. Pigs were then moved from the farrowing site to the nursery site where they were allotted to pens by group, gender, and size. They were housed at a density between 0.23 and 0.28 m^2/ pig and were allotted to each pen from the 3 groups. Pigs remained in nursery rooms for approximately 49 d and received a 4-stage nursery feeding program. The first 2 diets were pellets and purchased from a commercial company. The last 2 diets were formulated internally (Table 2). A feed budget was implemented by feeding strict amounts of each diet phase: 0.23, 2.0, 5.4 and 18 kg/pig respectively. After pigs completed their nursery period, they were moved to adjacent finish facilities. The nursery and finish buildings were separated by a weighing area.

Each pig was individually weighed (model 700, True Test; Auckland, New Zealand) and ear tagged with an electronic transponder for feed intake recording by Feed Intake Recording Equipment (FIRE) feeders (Osborne Industries; Osborne, KS). When pigs were weighed on test, real-time ultrasound (RTUS) back fat was determined using an Aloka real-time ultrasound meter (model SSD 500 B Aloka, Wallingford, CT) at the first rib, last rib, and last lumbar vertebrae by a trained technician. Loin depth was estimated at the last lumbar vertebrae. The pigs were fed using a 4-phase finish program (Table 3); diets were corn-soy based and met PIC USA specifications for commercial PIC pigs (PIC USA, 1999) that exceeded NRC specifications (NRC, 1998). Pigs were individually weighed every 14 d until they reached an average weight of 122.5 kg.

The finish building consisted of 6 rooms of pens with 4 pens per side in each room. Fifteen castrates or 15 gilts were allotted to each pen. Pigs from the 3 groups were randomly allotted to each pen by size and gender. Differences in body weight within a pen between the lightest and heaviest pigs did not exceed 6.8 kg at placement. A total of 698 pigs were started on test. Temperature in the finish building was maintained at 21 ± 0.5 ^{o} C. Feed Intake Recording Equipment (FIRE) feeders were used to record daily feed intake for each pig. Two pens of pigs shared the same FIRE feeder with a swinging fence-line gate between the 2 pens. The position of the gate was changed once per week to control which pen of pigs had access to the FIRE feeder during the week.

Age at 125 kg was calculated as: Age + {(125 – off-test weight) x (age – 41.84)} / age. Pigs that were removed early from test because of death, injury or illness were assigned a

value of "1" for the early removal variable and a "0" was assigned to all pigs that were weighed off test at the completion of the experiment.

The pigs were humanely slaughtered a commercial pork processing plant that was located in the Midwest. Hot carcass weight recorded for each carcass and a Fat-O-Meter (SFK Technology, Herlev, Denmark) was used to record back fat depth and loin depth for each carcass. From these variables carcass lean percentage was estimated using the following equation (PIC formula): Lean % = 58.91586 – (0.56074*back fat, mm) + (0.10585*loin depth, mm).

To investigate the relationship between 20-d weaning weight and age to 125 kg body weight, growth rate, feed intake, feed conversion, and carcass lean, data from only the **SR** and **14W** groups were used. Relative growth and other variables were similar for these treatments despite the population shift in weaning weight. The 2W group was excluded from this analysis because their performance was inferior due to the short time of being with the sow even though the nutritional restriction for growth was removed. The **SR** and **14W** populations performed relatively similar, with only a slight advantage to **SR** pigs in measures of long-term growth. For this reason, they were pooled with a total of 468 pigs being grouped into one of 6 weight classifications based on their weights at 20-d of age. Weight classifications were fitted to a normal distribution: 4.1 to 5.0 (9%), 5.0 to 5.9 (17%), 5.9 to 6.8 (25%), 6.8 to 7.7 (24%), 7.7 to 8.6 (16%), and 8.7 to 11.5 kg (9%). Least squares means were calculated for each weight classification and orthogonal polynomials were fitted to determine linear and quadratic responses for each trait. Pigs in the 4.1- to 5.0- kg classification were largely pigs from the **SR** group (91%) while pigs in the 8.7 to 11.5 kg classification were represented to a greater

extent by the **14W** group (70%). The other weight classifications consisted of a balanced mixture of both **SR** and **14W** pigs.

Statistical Analysis. SAS PROC MIXED (SAS, 2000) was used to complete the statistical analyses comparing differences among the **SR, 14W,** and **2W** groups for all traits except for survival rate, mortality, and early removal percentage. For these traits, SAS PROC GLIMMIX was used to complete the analyses. Source of variation accounted for in the analyses are presented in Table 4. Litter nested within sow line and farrowing group was considered a random effect and was the error term for testing group differences in the analyses completed using PROC MIXED. Group differences were tested using the PDIFF option in PROC MIXED and PROC GLIMMIX whenever there was a significant F-test for the "Group" source of variation in the Analysis of Variance. In all statistical models that included covariates, except those models used to analyze the carcass traits, separate linear and quadratic covariates were fit for each of the three groups. Statistical models used to estimate the effects of 20-d weight on growth, feed intake, and feed conversion during the finish period and on carcass composition were the same as those used to compare differences among the **SR**, **14W**, and **2W** groups.

For the estimation of the effects of 20-d weaning weight only data from the **SR** and **14W** groups were used. Each pig was assigned to 1 of 6 groups based on its 20-d weaning weight. Linear and quadratic orthogonal polynomials were fit to estimate group responses. The distribution of observations for all three groups is shown in Table 5. The least squares means by group for the pre-weaning though nursery, post-nursery (finishing), and RTUS and carcass traits are presented in Tables 6, 7, and 8 respectively.

RESULTS

Pre-weaning Performance. Pig birth weight did not differ among the 3 treatments (Table 6), however, pigs from the three groups differed ($P < 0.05$) in their weaning weight which is consistent with their weaning ages differences. Older groups (i.e., **14W** and **2W**) weighed 1.01 and 2.26 kg more, respectively, when compared to pigs in the SR group ($P < 0.05$). Pigs from the **2W** group weighed 1.25 kg more compared to pigs from the **14W** group ($P < 0.05$). These differences in weaning weight show that the different nutrition strategies (sow lactation length, MR) were successful in creating mean separated weight groups at weaning. Survival rates during the pre-nursery period (20 ± 0.2 d) were 87.9, 81.5, and 78.0 % for the **14W**, **SR**, and **2W** groups, respectively.

Nursery Performance. Pigs from the **14W** group grew 35 g/d faster ($P < 0.05$) during the nursery period compared to pigs from the **2W** group (Table 6). Despite the 2.2 kg advantage of the **2W** group, the growth rate for **SR** pigs was numerically greater (569 vs. 547 g/d) than the **2W** group.

Post-Nursery Performance. Pigs averaged 69 d of age when they were placed in the grow-finish site to evaluate their growth performance. Pigs from the **14W** and **2W** groups were 2.3 kg and 2.6 kg heavier, respectively than the **SR** treatment to begin the test (Table 6). However, rate of gain for the **SR** and **14W** groups (Table 7) was identical (816 g/d) for the entire period but ADG for the **2W** group was 31 g/d poorer ($P < 0.05$).

The ADG for the **SR** and **14W** groups was greater ($P < 0.05$) during finishing period when compared to the pigs from the **2W** group. Pigs from the **14W** group reached a body

weight of 125 kg 3.0 d sooner than **SR** pigs ($P < 0.05$) and **SR** pigs were not different from **2 W** pigs despite weighing 2.2 kg less at weaning. Pigs from the **SR** and **14W** groups grew 24 and 20 g/d faster, respectively, during the finishing period (33 kg to 125 kg) than **2W** pigs ($P < 0.05$). Feed conversion of **SR** pigs was improved ($P < 0.05$) over **14W** pigs and was numerically better than **2 W** pigs during the finishing period.

The **SR** pigs had 2.0 and 1.7 mm more Fat-O-Meter (FOM) loin depth ($P < 0.05$) than loins of the **2W** and **14W** pigs, respectively, at harvest (Table 8). The **SR** pigs' carcass lean percentage was 0.7 % greater ($P < 0.05$) than that of the **2W** pigs (55.0 vs. 54.3% respectively, Table 8).

Impact of 20-d weaning weight. Since progeny long-term growth and composition of growth was different for the **2W** pigs as compared to **SR** counterparts, this analysis was confined to pigs that remained with the sow for at least 14 d (**SR** and **14W**). A summary of nursery and finishing performance is provided in Table 9. Nursery ADG increased linearly ($P < 0.001$) with increasing weaning weight. Grow-finish final weight increased linearly ($P < 0.001$) with increasing weaning weight. Age to 125 kg was reduced (quadratic, $P < 0.05$) with increasing weaning weight. Pigs that weighed between 4.1 and 5.0 kg required 176.8 d to reach 125 kg, which was 8.0 d more compared to pigs weighing between 5.0 and 5.9 kg at 20 d of age. An extra 15.9 d were required when compared to pigs that weighed between 8.7 and 11.5 kg. Finish ADG increased (quadratic, $P < 0.10$) with increasing weaning weight. Pigs in the 4.1 to 5.0-kg classification gained 886 g/d during the post-weaning period, which was 46 to 59 g/d less than pigs weighing 5.0 kg or more. Estimates of the linear and quadratic coefficients

were 4.176 g/d ($P < 0.05$) and -2.326 g/d^2 ($P < 0.10$), respectively. Feed intake was increased (linear, $P < 0.05$) with increasing weaning weight.

Real-Time Ultrasound (RTUS) back fat and loin depth and carcass data are shown in Table 10 for the 6 weaning weight classifications. The RTUS loin depth decreased (-0.334 mm) (linear, $P < 0.01$) with increasing weaning weight. Hot carcass weight increased (linear, $P < 0.001$) with increasing weaning weight. The FOM on carcass loin depth decreased (-0.24 mm) (linear, $P < 0.05$) with increasing weaning weight.

DISCUSSION

The most important and unexpected, finding of this trial was that the longer the sow nursed the litter, the better her progeny tended to perform in ADG, gain: feed, viability (mortality plus illness), loin and fat depth. This is consistent with the work reported by Main and co-workers (2004) who weaned litters at 12, 15, 18, or 21 d of age. They found that increasing weaning age increased overall (linear, $P < 0.001$) weaning-to-finish ADG, weight sold per pig weaned and decreased (linear, $P < 0.03$) mortality rate. They concluded that increasing weaning age from 12 to 21.5 d of age increased weight sold per pig weaned by 1.80 ± 0.12 kg for each day increase in weaning age. The study suggested that increasing weaning age up to 21.5 d can be an effective management strategy to improve weaning-to-finish growth performance in multisite pig production.

Another important finding was the failure of the **2W** pigs to completely retain the substantial weaning weight advantage over the lighter **SR** and **14W** pigs throughout the post-weaning period. Early-weaning at less than 10 d of age followed by removal of pigs to a

second isolated site is known to reduce immunological stress resulting in improved growth and feed efficiency (Johnson, 1997; Maxwell, 1999). Cabrera et al. (2002) applied this principle to small pigs at birth. We observed that the size of small pigs (less than 1.13 kg on average) can be reclaimed by separating them from their dam at the age of 2 d post birth by placing them in a controlled isolated nursery environment receiving an unlimited amount of a properly fortified, formulated milk powder. These small pigs showed greater potential for growth and livability than that observed if they remained with the sow. This management practice allowed the weaning of more high-quality pigs beyond the rearing capacity of the sow. However when these small pigs were comingled with their siblings in the nursery and finishing phases, we observed lower survival rate than when reared separately. We concluded that they had a different health status (naïve) when compared to their sow reared siblings. When considered in light of the present experiment, length of time spent with the sow is important to long-term viability even if progeny remained in the pen from which their mother was removed from. A separate pig flow within a specific production system is best when rearing early-weaning small pigs.

The undeniable fact of this experiment is that long-term whole-body and carcass growth were benefited by piglet spending 20 d with the sow when compared to either 14 or 2 d. The influence of the sow beyond the colostrum period and even to 14 d of age, is significant. The biological basis for the far reaching effects of the sow, on the performance of her progeny is not clear.

The North American swine industry reduced nursing length during the 1990's from about 21 d to as little as 12 to 14 d because of proposed health advantages (segregated early

weaning). However, this procedure compromised long-term piglet growth and viability. These effects result in a known compromise in the number of pigs weaned per sow per year.

One issue with early weaning is the impact that the length of the lactation period may have on cellular immunity in the pig. Blecha et al. (1983) have shown that early weaning might compromise cellular immune function in young pigs. In their study, early-weaned pigs had suppressed in vivo and in vitro cellular immune responses. The capability of lymphocytes to undergo blastogenesis in response to phytohemagglutinin was decreased in early-weaned pigs. This requires further investigation because the physiological and immunological mechanism(s) responsible for weaning-impaired cellular immunity are unknown.

Moeser et al. (2006) investigated the impact of weaning on gastrointestinal health in the pig and to assess the role of stress signaling pathways in this response. They weaned 19-d-old pigs compared to age-matched, un-weaned control pigs and assessed mucosal barrier function and ion transport in jejunal and colonic tissues mounted on Ussing chambers. They found that weaning caused marked disturbances in intestinal barrier function, as demonstrated by significant reductions in transepithelial electrical resistance (TER) and increases in intestinal permeability to [^{3}H] mannitol in both jejunum and colon. These two characteristics are an indication of a poor and leaky epithelial barrier. In a similar study, Moeser et al. (2007) also found the same results when comparing piglets weaned at 19 d of age to 28-d weaning; proper barrier function favored the latter.

The advantages of **SR** pigs over the **2W** pigs might be explained by components in the sow's milk and controlled pathogen exposure, which could potentially improve their progenies' long-term health when compared to milk replacement. In humans, breast milk

oligosaccharides have been shown to selectively stimulate the growth of Bifidobacteria and Lactobacilli in the intestine of infants (Moreno, 2008). The intestinal microflora of breast-fed infants is an important physiological factor in gut function and the development of the immune system. Hosea et al. (2008) found that breast milk contains (1) various antimicrobial substances, (2) factors that promote immune development, (3) constituents that promote tolerance or priming of the infant immune system as well as anti-inflammatory components.

In humans, breastfeeding has proven to have a long-lasting effect on the baby's future health (Lowdon, 2008). Bilenko et al. (2008) has shown that partial breastfeeding has protective effects against enteric infection (*Cryptosporidium spp, Campylobacter spp*, ear infections and asthma) and associated morbidity. Bosnjak and Grguric (2007) have compiled data from 2001 and 2006 indicating breastfeeding is likely to protect against later obesity, type 1 diabetes, celiac disease, inflammatory bowel diseases and childhood cancer. These findings are consistent with those found by Schack-Nielsen and Michaelsen (2006). Loland et al. (2007) indicated that human milk possibly affects components of the metabolic syndrome and lowers the risk of autoimmune diseases. Given the established health benefits in human milk we assume that the same benefits are inherent in sows when nursing their progeny.

The 3 rearing lactation strategies successfully produced the desired weaning weight subpopulations (**SR** = 4.1-6.6 kg, **14W** = 6.8 to 8.4 kg and **2W** = 8.6+ kg). This approach avoids the confounding problem of weaning weights that are a function of birth weight (Schinckel et al., 2007). In our case, birth weight represented less of a bias; however, nursing length needed to be re-examined. The latter could be controlled by eliminating the most disparate treatment (**2W**).

The **14W** strategy represents a commercially feasible result of genetically-increased sow milk output. The **2W** strategy represents an alternative scenario in which the genetic potential for growth by the piglet is not constrained by the mother. It was possible that one or both of the strategies (**14W** and **2W**) might be compromised if the sow (milk, fecal microbe shedding) is important to life-time immune competence.

Early weaned groups (**2W** and **14W)** received unlimited access to the MR for 18 and 6 d, respectively, which created an average increase of 2.2 and 1.0 kg/pig compared to **SR** pigs. This is consistent with other reports showing greatly accelerated growth of young pigs fed liquid milk products (Zijlstra et al., 1996; Azain, 1997; Heo et al., 1999). The growth rate of the neonatal pig is largely determined by sow milk nutrient output (Noblet and Etienne, 1989). Boyd et al. (1995) proved that the nursing pig has substantially greater growth potential than is being realized because milk secretion is inadequate for maximum growth. The sow appears to be limiting maximum pig weight gain as early as 8 d after farrowing (Harrell et al., 1993). They found that in pigs with unlimited nutrient supply, the average growth rate is 400 g/d from birth to 21 d of age. This is 170 g/d greater than a typical growth rate for sow-reared pigs (230 g/d). More important is the observation that the rate of growth increased in a linear manner to an average of 521 g/d between 17-21 d of age.

The accelerated growth of ad-libitum feeding a liquid diet early in the neonatal pigs' life is well documented (Harrell et al., 1993; Boyd et al., 1995; Kim et al., 2001). Our study points out that although the sow limits the biological maximum for growth, she is to this point indispensable to long-term growth and viability. The **14W** pigs were able to maintain their

weaning weight advantage over the **SR** pigs from weaning to 125 kg (3 d less required); however composition of gain and viability numerically favored **SR** pigs.

Spencer et al. (2003) reported that early-weaned (d 14) piglets (under extreme heat stress) fed a MR were able to maintain their weaning weight advantage though the 47-d nursery period. In their study, primiparous and multiparous sows were exposed to either a hot or thermo neutral (TN) temperature (32^{o} and 21^{o}, respectively) and weaned them either at 14 d or 19 d of lactation. The objective of this regimen was to prevent extreme maternal weight loss by early weaning. When sows were removed at 14 d of lactation, pigs were allowed to consume milk supplement ad libitum. The growth rate of piglets in the TN and hot environment increased to 605 g/d and 717 g/d, respectively during these 5 d. The 1.8 kg/pig advantage at weaning increased to 3.9 kg/piglet at the end of 47 d nursery. This increase (2.2:1) is consistent with the increase obtained in this study (2.3:1). Unfortunately, pigs were not grown to harvest, which is important to understanding the outcome.

In our study, the weaning weight advantage of **14W** pigs over **SR** controls (1.0 kg/pig) was maintained to harvest, because ADG was similar from weaning to harvest (816 g/d). This difference in weaning weight did not invoke a more rapid growth rate to harvest. The net effect was to reduce the time to reach 125 kg whole-body weight for **14W** pigs.

Our study showed that composition of gain was improved by a longer lactation. There was no difference in back fat or loin depth between milk-fed pigs (**2W** and **14W**) and **SR** pigs at the end of the nursery period. However at harvest, **SR** pigs had greater real-time ultrasound and FOM loin depth when compared to **2W** and **14W** pigs. The **SR** pigs were 0.7% leaner than **2W** pigs. The **SR** pigs had numerically greater % lean than **14W** pigs.

Using data from **SR** and **14W** subpopulations of pigs, the advantage of weaning heavier pigs became clear. We were also able to confirm, once again, the relationship between birth weight and weaning weight. Heavier pigs at 20 d of age did maintain their advantage (ADG, ADFI) and required fewer d to reach market weight. Mahan and Lepine (1991) observed that gains and feed intakes were greater as weaning weight increased during both the nursery and the finishing periods. In our study, pigs weighing between 5.0 and 5.9 kg at 20 d of age were able to reach 125 kg body weight 8 d sooner than pigs weighing between 4.1 and 5.0 kg. This is consistent with Azain's work (1997). He found that pigs weighing on average 5.65 kg reached 104 kg of bodyweight 7 d sooner than those weighing 4.5 kg. Mahan et al. (1998) fed a complex nursery diet for 1, 2, or 3 wk to 23-d-old weaned pigs of 2 weaning weights (5.5 or 7.5 kg). Pigs with heavier weaning weight reached 105 kg BW approximately 8 d sooner than those with lower weaning weight. He concluded weaning heavier pigs seemed to have greater effect on post-weaning performance than the feeding duration of the complex nursery diet. Unlike our results, they were able to show better feed utilization on heavier weaned pig in the growing period. In a similar study, Mahan et al. (1993) found that pigs weighing between 6.8 and 8.2 kg reached 105 kg approximately 10 d earlier than pigs weighing between 4.1 and 5.5 kg.

The concern with each of these studies is that birth weight is confounded with weaning weight. Further, the growth curve for smaller birth weight pigs does not achieve the same level as for pigs with greater birth weight.

We propose that the sow influences her progeny's performance beyond the colostrum period and in a profound way. Based on what is known in human nutrition, the maternal

influence of milk is important to the development of immune competence beyond the contribution of colostrum. This trial confirms the growth potential of the neonatal pig is significantly beyond the sow's milking ability for a 20-d lactation period (**2W** group) and that pigs that weigh less than 5.0 kg at weaning represent the greatest marginal opportunity to decrease days to harvest (8 d more to achieve 125 kg of body weight or weigh 3.3 kg less than 5.5 kg pigs). Segregated early weaning technology (SEW) not only failed to deliver improved health in practice but compromised piglet whole-body and composition of growth and viability, especially under situations of high immune stress. These data can be used in economic models to determine the (1) value of lactation length on progeny performance to harvest, and (2) the value of genetic improvements in milk production. The increment of greatest opportunity appears to lie between 4.6 and 5.5 kg at 20 d of age. The bottom 9% of the pigs (4.1 to 5.0 kg) imposes significant cost to a system because of lower market weights since increased time to achieve target weight is a poor financial option.

Table 1. Nutrient composition of the acidified milk replacer (calculated values) fed to piglets.

Nutrients	Units	Concentration
Crude protein	%	24.10
Fat	%	18.10
Fiber	%	0.02
Ash	%	7.74
Carbohydrates	%	46.92
Dry Matter	%	96.88
Energy		
ME Swine	Kcal/kg	3868
Amino acids		
Alanine	%	1.04
Arginine	%	0.80
Cystine	%	0.55
Glycine	%	0.50
Histidine	%	0.58
Isoleucine	%	1.23
Leucine	%	2.26
Lysine	%	2.10
Methionine	%	0.49
Phenylalanine	%	0.72
Threonine	%	1.54
Tryptophan	%	0.41
Tyrosine	%	0.72
Valine	%	1.32
Minerals		
Calcium	*%*	0.91
Phosphorus	*%*	0.73
Sodium	*%*	0.73
Chlorine	*%*	0.06
Magnesium	*%*	0.16
Potassium	*%*	1.22
Sulfur	%	0.47
Cobalt	mg/kg	0.85
Copper	mg/kg	254.36
Iodine	mg/kg	3.56

Table 1. Continued.

Iron	mg/kg	160.40
Manganese	mg/kg	44.45
Zinc	mg/kg	96.0
Selenium	mg/kg	0.357
Vitamins		
Vitamin A	IU/kg	99,000
Vitamin D_3	IU/kg	33,000
Vitamin E	IU/kg	220
Vitamin K	mg/kg	1.83
Biotin	mg/kg	0.31
Vitamin B_{12}	mg/kg	0.09
Folic Acid	mg/kg	1.12
Niacin	mg/kg	73.35
Pantothenic Acid	mg/kg	57.05
Pyridoxine	mg/kg	9.48
Riboflavin	mg/kg	26.99
Thiamine	mg/kg	9.09
Choline	g/kg	1.30
Vitamin C	mg/kg	170.70
Medication		
Oxytetracycline	mg/kg	50.00
Neomycin	mg/kg	100.00

Table 2. Composition of nursery diets for the growth periods of 7 to 11 kg and 11 to 27 kg (as-fed basis)[1].

Ingredients, %	Nursery 7 to 11 kg	Nursery 11 to 27 kg
Corn, 8.5	57.38	66.03
Soy Meal 48	19.65	25.0
Fish Menhaden 60	9.20	2.21
Fat, animal/vegetable blend	4.37	2.90
L-Lysine	0.15	0.40
DL-Methionine	0.07	0.17
Limestone	0.10	0.44
Dicalcium 18P 21Ca	0.61	1.19
Salt	0.44	0.51
Med. Neo-Terramycin 10-10	1.00	0.10
Base Mix Nursery 7-11[2,3]	7.04	-
L-Threonine	-	0.10
Copper Sulfate	-	0.08
VTM Premix[c]	-	0.86
Vitamin E 20,000	-	0.02
Calculated Composition		
NRC ME, Kcal/kg	3440	3440
Total Lysine, %	1.45	1.38
Calcium, %	0.80	0.70
Phosphorus, %	0.73	0.64

[1]Diet compositions of the early weaned and phase 1 diets (pigs weighing between 12 to 16 lbs of body weight) are not displayed and were purchased from Hubbard Feeds, Inc. (Mankato, MN). Early weaned diets (designated for pigs less than 5.5 kg) were budgeted to provide 0.2 kg/pig and the phase 1 diet (5 to 7 kg pigs) was budgeted to provide 2.0 kg/pig.

[2]Base Mix Nursery 7 to 11 includes the vitamin and trace mineral and other ingredients purchased from Hubbard Feeds, Inc.

[3]Basemix and premix supplied per kg of diet: Vitamin A, 9923 IU; vitamin D_3, 1654 IU; vitamin E, 77 IU; vitamin K (menadione activity), 4.0 mg; riboflavin, 9.9 mg; D-pantothenic acid, 33 mg; niacin, 55 mg; vitamin B_{12}, 44 µg; D-biotin, 0.28 mg; folic acid, 1.0 mg; thiamine, 3.3 mg; pyridoxine, 5.5 mg; Zn, 275 mg ($ZnSO_4$); Cu, 33 mg ($CuSO_4$); Fe, 220 mg ($FeSO_4$); Mn, 99 mg ($MnSO_4$); I, 1.5 mg (EDDI); and Se, 0.30 mg (Na_2Se).

Table 3. Composition of the growth-finish diets (as-fed basis)[1].

Ingredients, %	Fin 27-45 kg	Fin 45-73 kg	Fin 73-95 kg	Fin 95-118 kg
Corn, 8.5	60.69	60.04	68.32	73.17
Soy Meal 48	28.71	23.98	16.29	11.72
Wheat Midds	5.00	10.00	10.00	10.00
Fat, A-V	2.59	3.15	2.59	2.31
L-Lysine, HCl	0.15	0.09	0.18	0.20
DL-Methionine	0.06	-	-	-
L-Threonine	0.03	0.02	0.06	0.09
Limestone	0.69	0.70	0.60	0.64
Dicalcium 18P 21Ca	0.82	0.75	0.69	0.61
Salt	0.46	0.46	0.46	0.46
Copper Sulfate	0.07	0.06	0.06	0.06
VTM Premix[2]	0.75	0.75	0.75	0.75
Calculated				
NRC ME, kcal/kg	3300	3300	3300	3300
Total Lysine, %	1.21	1.05	0.91	0.79
Calcium, %	0.62	0.60	0.52	0.50
Phosphorus, %	0.57	0.57	0.53	0.49

[1] All diets were fed in a meal form and were switched with the average weight of the pigs.
[2] Supplied per kg of diet: Vitamin A, 9900 IU; vitamin $D_{3,}$ 1760 IU; vitamin E, 66 IU; vitamin K (menadione activity), 4.4 mg; riboflavin, 9.9 mg; D-pantothenic acid, 100 mg; niacin, 44 mg; vitamin $B_{12,}$, 37.4 µg; D-biotin, 0.22 mg; folic acid, 1.32 mg; choline, 0.66 g; thiamine, 2.2 mg; pyridoxine, 3.3 mg; Zn, 125 mg ($ZnSO_4$); Cu, 15 mg ($CuSO_4$); Fe, 100 mg ($FeSO_4$); Mn, 50 mg ($MnSO_4$); I, 0.35 mg (EDDI); and Se, 0.30 mg (Na_2Se).

Table 4. Sources of variation which were included in statistical models.

Source of variation	Degrees of Freedom	Birth Wt., 20-D Wt., Age Entering Nursery	Nursery ADG	Wt. and Age Entering Finish	Ultrasound Back fat and Loin Depth Entering Finish	[9] Wt, ADG, ADFI and G:F	[10] ADG	Age at 125 kg., Off-Test Wt.	Ultrasound Back fat and Loin Depth	Survival Rate, Death Loss, Early Removal Percentage	Carcass Traits
Breed	2	X[1]	X	X	X	X	X	X	X	X	X
Group	2	X	X	X	X	X	X	X	X	X	X
Sex	1	X	X	X	X	X	X	X	X	X	X
Harvest	4	-[2]	-	-	-	-	-	-	-	-	X
Age	1	X[3]	-	X[4]	-	X[6]	-	-	-	-	-
Age	1	-	-	-	-	X[6]	-	-	-	-	-
TRT x	3	-	X	-	-	-	X	-	-	-	-
TRT x	3	-	-	X[5]	X	X[7]	-	-	-	-	-
TRT x	3	-	-	-	-	-	-	-	X	-	-
TRT x	3	-	-	-	-	-	-	-	X[8]	-	-
Hot	1	-	-	-	-	-	-	-	-	-	X
Random											
Litter		X	X	X	X	X	X	X	X	-	X

[1] X effect included in statistical model.
[2] Effect not included in statistical model.
[3] Linear covariate included only in statistical analysis of 20-d weight.
[4] Linear covariate included in statistical analysis of weight entering finish.
[5] Linear covariates included in statistical analysis of age entering finish.
[6] Linear and quadratic covariates included in analyses of weight after 4, 8, and 12 weeks in the finish.
[7] Linear covariates included in statistical models in analyses of average daily gains after 4, 8, and 12 weeks in the finish.
[8] Quadratic linear covariate included in statistical model in analysis of ultrasound loin depth.
[9] After 4, 8, and 12 wks in finish and nursery – finish test period.
[10] From nursery to 4, 8, and 12 wks in finisher and finish test period.

Table 5. Number of observations by treatment group for growth and carcass traits of pigs that were sow reared (SR), wean at 14 d of age (14W) and wean at 2 d of age (2W).

Trait	SR	14W	2W
Birth weight	367	330	337
Weight entering nursery	299	290	263
Age entering nursery	299	290	263
Nursery ADG	244	226	228
Finish Growth Assay			
Weight	244	226	228
Age	244	226	228
[1]RTUS back fat at first rib and last lumbar	244	226	228
RTUS loin depth	244	226	228
28-d Weight and ADG	243	224	226
56-d Weight and ADG	242	222	222
84-d Weight and ADG	238	221	216
Off test			
Weight	233	219	210
Age off test	233	219	210
Age to 125kg	233	219	210
ADG	233	219	210
ADG from 20 d of age	233	219	210
Daily feed intake	178	179	188
Feed conversion	178	179	188
% completing test	233	219	210
RTUS back fat at first rib and last lumbar	233	219	210
RTUS loin depth	233	219	210
% dead	233	219	210
% early removals	233	219	210
Carcass traits			
Hot carcass weight	233	210	210
Carcass yield	233	210	208
Fat-O-Meter back fat	232	204	207
Fat-O-Meter loin depth	232	204	207
Lean percentage	232	204	207

[1]Real-time ultrasound (RTUS).

Table 6. Least square means for performance traits from pre-weaning though nursery pigs that were sow reared (SR), wean at 14 d of age (14W) and wean at 2 d of age (2W).

Trait	**SR**	**14W**	**2W**	**Pooled SEM**
Number pigs placed post-farrow	367	330	337	
Number pigs weaned	299	290	263	
Survival rate, %	81.5	87.9	78.0	
Pig birth wt., kg	1.60	1.58	1.67	0.04
Pig weaning weight, kg	6.49[c]	7.50[b]	8.75[a]	0.13
Age at weaning, d	19.5[b]	20.4[a]	21.0[a]	0.2
Nursery ADG[1], g / d	569.0[ab]	582.0[a]	547.0[b]	7.7
Nursery final wt., kg	33.5[b]	35.8[a]	36.1[a]	0.43
Nursery final age, d	70.4[a]	68.8[b]	68.1[b]	0.5

[a, b, c] Within a row, means without a common superscript differ ($P < 0.05$).

[1] During the nursery period 3 **SR** pigs, a **14W** pig, and a **2W** pig died.

Table 7. Least square means for performance traits during the finishing period of growth (33 kg to 125 kg) for pigs that were sow reared (SR), wean at 14 d of age (14W) and wean at 2 d of age (2W).

Trait	SR	14W	2W	Pooled SEM
1 to 28 d				
Initial Weight[1], kg	58.0[b]	58.9[a]	58.9[a]	0.63
ADG, g/d	836	834	825	12
29 to 56 d				
Initial Weight, kg	83.5[b]	86.7[a]	85.3[ab]	0.8
ADG, g/d	913[a]	913[a]	885[b]	10
57 to 84 d				
Initial Weight, kg	110.2[b]	112.6[a]	111.3[ab]	0.8
ADG, g/d	927	922	904	8
Final Weight, kg	123.5	124.8	123.9	0.6
Final Age (unadjusted for weight), d	166.6	164.7	165.2	0.8
Age to 125kg, d	167.7[b]	164.7[a]	166.1[ab]	0.9
Finish ADG, g/d	936[a]	932[a]	912[b]	8
ADG from 20 d of age, g/d	816[a]	816[a]	785[b]	6
Finish Daily feed intake, kg/d	2.22[b]	2.32[a]	2.20[b]	0.03
Gain:Feed	0.44[a]	0.42[b]	0.42[b]	0.01
% Completing Test	98.7	97.0	95.8	1.1
% Dead	1.1	2.0	3.3	1.0
% Early Removals[2]	0.2	1.0	0.9	0.5

[a, b, c] Within a row (or column), means without a common superscript differ ($P < 0.05$).

[1] Age of pigs was used as covariate when determining the final weights for the different analyzed periods.

[2] Reasons for removals were lameness, unthriftiness, etc.

Table 8. Least square means of real-time ultrasound (RTUS) and fat-o-meter (FOM) measures on whole-body and carcass data for pigs that were sow reared (SR), wean at 14 d of age (14W) and wean at 2 d of age (2W).

Trait	SR	14W	2W	Pooled SEM
Initial RTUS back fat at first rib, last lumbar, mm	10.5	10.4	10.6	.2
Initial RTUS loin depth, mm	29.3	29.1	28.6	.3
Final RTUS back fat at first rib and last lumbar, mm	19.2	19.2	19.4	.4
Final RTUS loin depth, mm	55.6[b]	53.8[a]	53.7[a]	.5
Hot carcass weight, kg	91.1	91.9	92.1	.6
Carcass Yield, %	73.7	73.5	73.7	.1
FOM fat depth, mm	17.7	18.1	18.3	.4
FOM loin depth, mm	57.9[b]	56.2[a]	55.9[a]	.6
Calculated Lean percentage[1], %	55.0[b]	54.7[ab]	54.3[a]	.3

[a,b,c] Within a row, means without a common superscript differ ($P < 0.05$).

[1]Lean % equation = 58.91586 – (0.56074*back fat, mm) + (0.10585*loin depth, mm).

Table 9. Least square means for the combined data of SR and 14W groups of pigs for nursery and finishing phases of growth.

	Weaning Weight Classification, kg/pig								
Traits	**4.1 to 5.0**	**5.0 to 5.9**	**5.9 to 6.8**	**6.8 to 7.7**	**7.7 to 8.6**	**8.7 to 11.5**	**Linear**	**Quadratic**	**Pooled SEM**
No. of Pigs	42	78	116	112	78	42			
Population, %	9	17	24	24	17	9			
Pig birth weight, kg	1.43	1.43	1.49	1.60	1.71	1.85	0.043***	0.011†	0.05
Weaning age, d	19.1	19.6	20.0	20.2	20.4	21.0	0.175***	-0.002	0.3
Nursery ADG, g/d	507	530	549	579	593	607	10.254***	- 0.730	10
Test Data									
Final weight, kg	119.4	122.7	125.1	123.9	125.8	126.5	0.623***	-0.183	1.0
Age off test, d	172.5	167.0	167.9	164.1	163.7	161.9	-0.955***	0.164	1.2
Age to 125 kg, d	176.8	168.8	167.8	164.8	162.9	160.9	-1.432***	0.319*	1.3
ADG, g/d	886	939	936	932	939	945	4.176*	-2.326†	11.3
ADG from 20 d, g/d	752	796	804	815	825	831	7.063***	-2.126*	8
Daily feed intake, kg/d	2.18	2.30	2.25	2.29	2.27	2.47	0.020*	0.006	0.05
Gain: Feed	0.42	0.41	0.42	0.41	0.42	0.39	0.010	0.007	0.01
% Completing test	97.9	99.3	97.0	97.5	99.1	97.9	-0.005	0.027	1.9
% Dead	2.1	.8	3.1	1.5	1.0	.0	-0.186	-0.127	1.7

† $P < 0.10$.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$

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Table 10. Least square means for the combined data of SR and 14W groups for real-time ultrasound (RTUS), fat-o-meter (FOM) and carcass measures of pigs.

	Weaning Weight Classification, kg/pig								
Traits	**4.1 to 5.0**	**5.0 to 5.9**	**5.9 to 6.8**	**6.8 to 7.7**	**7.7 to 8.6**	**8.7 to 11.5**	**Linear**	**Quadratic**	**Pooled SEM**
RTUS back fat at shoulder, mm	23.0	21.0	21.7	21.9	21.2	22.8	-0.0003	0.144*	0.5
RTUS back fat at last lumbar, mm	16.3	15.4	16.4	16.8	16.7	16.4	0.065	- 0.017	0.4
RTUS loin depth, mm	55.5	56.1	55.1	54.4	52.7	53.0	-0.336**	-0.054	0.7
% dead	2.1	.8	3.1	1.5	1.0	0.0	-0.186	-0.127	1.7
Carcass data									
Hot carcass weight, kg	88.6	90.6	91.9	91.7	91.9	94.4	0.471***	-0.028	0.8
Carcass yield, %	74.3	73.7	73.4	73.6	73.3	73.9	-0.048	0.071**	0.2
FOM back fat, mm	17.9	16.8	17.7	18.3	17.8	18.8	0.112	0.058	0.7
FOM loin depth, mm	57.4	58.1	57.6	56.4	56.1	55.4	-0.242*	-0.081	0.8
Carcass lean percent, %	54.7	55.5	55.0	54.9	54.8	54.1	-0.078	-0.065†	0.3

† $P < 0.10$.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$

LITERATURE CITED

1. Azain, M.J. 1997. Nutrition of the young pig, use of liquid diets. In: Proc. 13th Annual Carolina Swine Nutr. Conf. Raleigh, NC pp 1-14.
2. Bilenko, N., R. Ghosh, A. Levy, R.J. Deckelbaum and D. Fraser. 2008. Partial breastfeeding protects Bedouin infants from infection and morbidity: prospective cohort study. Asia Pac. J. Clin. Nutr. 17 (2):243-249.
3. Blecha, F., D.S. Pollhman, and D.A. Nichols. 1983. Weaning pigs at an early age decreases cellular immunity. J. Anim. Sci. vol. 56, no. 2.
4. Bosnjak, A.P. and J. Grguric. 2007. Long-term health effects of breastfeeding. Lijec Vjesn 129 (8-9):293-298.
5. Boyd, R.D., R.S. Kessinger, R.J. Harrell, and D.E. Bauman. 1995. Nutrient uptake and endocrine regulation of milk synthesis by mammary tissue of lactating sows. J. Anim. Sci. 73 (Suppl. 2): 36-56.
6. Cabrera, R., R.D. Boyd, J. Mencke, J. Vignes, and J. Mauney. 2002. PICweaning Plus Technology: A paradigm shift in neonatal piglets care. PIC USA Technical Memo # 267. Franklin, KY 42134.
7. FASS. 1999. Guide for the care and use of agricultural animals in agricultural research and teaching. 1st rev. ed. Fed. Anim. Sci. Soc., Savoy, IL.
8. Harrell, R.J., M.J. Thomas, and R.D. Boyd. 1993. Limitations of sow milk yield on baby pig growth. Page 156 in Proc. Cornell Nutr. Conf. Ithaca, NY.

9. Heo, K.N., J. Odle, W. Oliver, J.H. Kim, I.K. Han, and E. Jones. 1999. Effects of milk replacer and ambient temperature on growth performance of 14-day-old early-weaned pigs. Asian-Aus. J.Anim.Sci. 12:908:913.
10. Hosea Blewett, H.J., M.C. Cicalo, C.D. Holland and C.J. Field. 2008. The immunological components of human milk. Adv. Food Nutr. Res. 54:45-80.
11. Johnson, R.W. 1997. Explanation why sick pigs neither eat well nor grow well. Proc. Carolina Swine Nutr. Conf. P. 49.
12. Kim, J.H., K.N. Heo, J. Odle, I.K. Han, and R.J. Harrell. 2001. Liquids diets accelerate the growth of early-weaned pigs and the effects are maintained to market weight. J. Anim. Sci. 79:427-434.
13. Loland, B.F, A.B. Baerug and G. Nylander. 2007. Human milk, immune response and health effects. Tidsskr Nor Laegeforen 127 (18):2395-2398.
14. Lowdon, J. 2008. Getting bone health right from the start: Pregnancy, lactation and weaning. J. Fam. Health Care 18 (4): 137-141.
15. Mahan, D.C. 1993. Effect of weight, split-weaning, and nursery feeding programs on performance responses of pigs to 105 kilograms body weight and subsequent effects on sow rebreeding interval. J. Anim. Sci. 71:1991-1995.
16. Mahan, D.C., and A.J. Lepine. 1991. Effect of weaning weight and associated nursery feeding programs on subsequent performance to 105 kilograms of body weight. J. Anim. Sci. 69: 1370-1378.

17. Mahan, D.C., G.L. Cromwell, R.C. Hamilton, and J.T. Yen. 1998. Evaluation of the feeding duration of a phase 1 nursery diet to thee-week-old pigs of two weaning weights. J. Anim. Sci. 76:578-583.
18. Main, R.G., S.S. Dritz, M.D. Tokach, R.D. Goodband and J.L. Nelssen. 2004. Increasing weaning age improves pig performance in a multisite production system. J. Anim. Sci. 82:1499-1507.
19. Maxwell, C.V. 1999. Nutrition and management of the early-weaned pig. Page 203 in Biotechnology in the Feed Industry. Proceedings of Alltech's 15th Annual Symposium. Nicholasville, KY.
20. Moeser, A., C.V. Klok, K.A. Ryan, J.G. Wooten, D. Little, V.L. Cook and A.T. Blikslager. 2006. Stress signaling pathways activated by weaning mediate intestinal dysfunction in the pig. Am. J. Physiol. Gastrointest. Liver Physiol. 292:173-181.
21. Moeser, A., K.A. Ryan, P.K. Nighot and A.T. Blikslager. 2007. Gastrointestinal dysfunction induced by early weaning is attenuated by delayed weaning and mast cell blockade in pigs. Am. J. Physiol. Gastrointest. Liver Physiol. 293:413-421.
22. Moreno Villares, J.M. 2008. Probiotics in infant formulae. Could we modify the immune response? An. Pediatr (Barc) 68 (3):286-294.
23. Morrow, M. 2004. What are the alternatives for saving our little piglets? International Pig Letter. January, 2004. Volume 23, No. 11c.
24. National Research Council (NRC). 1998. Nutrient Requirements for Domestic Animals. Tenth Revised Edition. National Academy of Sciences.

25. Pig Improvement Company (PIC). 1999. PIC Nutrient Requirements. Technical Memo. Franklin, KY.
26. Noblet, J., and M. Etienne. 1989. Estimation of sow milk nutrient output. J. Anim. Sci. 67: 3352.
27. SAS. 2000. The SAS Institute Inc.; Cary, NC.
28. Schack-Nielsen, L. and K.F. Michaelsen. 2006. Breast feeding and future health. Curr. Opin. Clin. Nutr. Metab. Care 9:289-296.
29. Schinkel, A.P., R. A. Cabrera, R.D. Boyd, S. Jungst, C. Booher, M.E. Johnston, P.V. Preckel and M.E. Einstein. 2007. Modeling the impact of birth and twenty-day body weight on the post-weaning growth of pigs. The Professional Animal Scientist 23: 211-223.
30. Spencer, J.D., R.D. Boyd, R.A. Cabrera, and G.L. Allee. 2003. Early-weaning to reduce tissue mobilization in lactating sows and milk replacement to enhance pig weaning weight during extreme heat stress. J. Anim. Sci. 81:2041-2052.

CHAPTER 3

BIRTH ORDER, BIRTH WEIGHT, SOW COLOSTRUM IgG AND PIGLET SERUM IgG CONCENTRATION AND THEIR EFFECTS ON NEONATAL SURVIVAL

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ABSTRACT

Intake of colostrum after birth is essential to stimulate intestinal growth and function, and to provide systemic immunological protection via absorption of Immunoglobulin G (IgG). IgG provides the majority of antibody-based immunity against invading pathogens. The birth order and weight of 745 piglets (from 75 litters) were recorded during a one-week period of farrowing. Only pigs weighing greater than 0.68 kg birth weight were chosen for the trial. Pigs with lower birth weight die to a greater extend in the first two days post-birth. Sow colostrum was collected during parturition, and piglets were bled between 48 and 72 h post-birth. Pig serum IgG and colostral IgG concentrations were determined by radial immunodiffusion. Data were analyzed using the GLM and REG procedures of SAS. Sow colostral IgG concentration explained 6% and piglet birth order accounted for another 4% of the variation observed in pig IgG concentration ($P < 0.05$). However birth weight had no detectable effect. Pig IgG concentration had both a linear ($P < 0.05$) and negative quadratic effect ($P < 0.05$) on % survival. Pigs with 1,000 mg/dl IgG or less (n=24) had a 67% survival; whereas, 39% of the pigs (n=247) had IgG concentrations between 2250 to 2500 mg/dl with 91% survival. Birth order had no detectable effect on survival, but birth weight had a positive linear effect ($P < 0.05$). Pigs weighing 0.9 kg (n = 107) at birth had a 68% survival rate, and those weighing 1.6 kg (n = 158; ~average birth weight) had an 89% survival. In conclusion, we found that sow colostrum IgG concentration and birth order can account for 10% of the variation of pig IgG concentration and that piglets with less than 1,000 mg/dl IgG serum concentration and birth weight of 0.9 kg at birth had low survival rate when compared to their larger siblings. Parity had a significant ($P < 0.001$) effect on IgG

concentration of sow's colostrum with parities two to eight having greater IgG concentration than parity one. In conclusion, the effective management of colostrum uptake in neonatal piglets in the first 24 h post-birth, may potentially improve piglets' survival from birth to weaning.

INTRODUCTION

Modern swine genotypes have been selected for increased litter size over the last 10-15 years which has resulted in lower piglet birth weights. Data (n = 6,039 piglets) collected from a large integrator sow farm showed that with an average litter size of 10 piglets an average birth weight of 1.6 kg, and sow parity ranging from 1-7, the percentage of piglets weighing less than 1.1 kg at birth was 19%. And, pre-weaning mortality was no less than 40% (PigCHAMP, 1998, 2002). These numbers will be higher if we consider the fact that litter size (born alive plus stillborn) has increased from 10.2 to 11.1 pigs per litter (PigCHAMP, 1998, 2007). Colostrum intake may be low in low birth weight piglets resulting in compromised health and elevated mortality. Indeed, Fix and See (2009) found that low birth weights pigs grew slower, were fatter, and were more likely to die at weaning. Ingestion of colostrum after birth is essential to stimulate intestinal growth and function and to provide systemic immunological protection via absorption of Immunoglobulin G (IgG). Klobasa and others (2004) investigated passive immunity (concentrations of serum immunoglobulins) on 603 neonatal piglets affected by birth order, litter size and parity in their first day of life. There was a significant effect of birth order because of the rapid changes in colostrum composition between birth of the first and that last piglet of each litter. The effect of birth weight was not detectable in IgG concentration and was different for the immunoglobulin

classes. There was no significant effect of litter size on passive immunization. Parity had a significant effect on IgG concentration in sow's colostrum with litter two to six having greater IgG concentration in their colostrum than parity one. These results are consistent with our findings. Machado-Neto and others (1987) found that a concentration of IgG of less than 10 mg/ml on postnatal d 1 has been associated with an increased pre-weaning mortality in piglets.

Our objective was to examine relationships among birth weight, birth order, IgG concentration and their correlation with piglet growth and mortality within a commercial swine farm environment.

MATERIALS AND METHODS

Pig and Sow Handling. All protocols were carefully supervised and approved by the corporate licensed veterinarians. All standard operating procedures for animal treatment and care were in agreement with published guidelines for animal care (FASS, 1999). The experimental animals were not subjected to prolonged restraint or surgical procedures and were humanely treated throughout the experiment. The farrowing of 82 sows (Monsanto Choice Genetics) was supervised for a period of one week in a commercial 1800-sow unit during the month of August 2008 in Smithfield, NC. Sows were allowed to farrow naturally. If they did not farrow by their due date (115 d gestation), they were induced with prostaglandin on d 116. At farrowing, piglets were dried, birth order recorded, weighed (Model S200 scale, Central City Scale; NE) and ear-tagged (Duflex tags, Digital Angel; MN) in both ears. The ear tags had INFECTA + GUARD®, a stud coating that carries the

bactericide *chlorhexidine gluconate*. The time of birth of each piglet was recorded. After processing, each piglet was positioned to the sow underline to encourage suckling. We aimed to place 11 piglets on every sow. If a sow had more than 11 piglets the remaining piglets were not used in the study. If a sow had less than 11 piglets, foster pigs were placed to complete 11 but the fostered piglets were not used in the study. Piglets that weighed less than 0.9 kg were not used in the study. Weaning age varied between 16 to 20 days and piglets were weighed individually using the same scale used to record their birth weight.

Sow Colostrum and Piglet Blood Collection. Sow colostrum was manually collected immediately after sows started farrowing. Colostrum was harvested in plastic cups, properly labeled with the sow ID and immediately refrigerated. Piglets were bled between 48 to 72 h post-birth using a 22 x 1.5" gauge needle and a 6 ml (13 x 100 mm) serum vacutainer tube (BD, Franklin Lakes, NJ). Blood samples were refrigerated and allowed to clot overnight. Serum was collected after centrifugation (10 min X 1300 g, IEC Centra GP8R, DJB Labcare Company, UK) and stored at -20 C until further analysis. Sow colostrum was centrifuged similarly and the defatted fraction containing the IgG was stored at -20 C until further analysis.

Immunoglobulin G Analysis. A radial immunodiffusion assay was used to determine IgG content in the piglet serum and sow colostrum (Fahey and McKelvey,1965; Mancini et al., 1965). Radial immunodiffusion is based on the diffusion of antigen from a circular well radial into a homogenous gel containing specific antiserum for a particular antigen (in this case, anti porcine IgG). A circle of precipitated antigen and antibody forms, and continues to grow until equilibrium is reached. The diameters of the rings are a function of antigen

concentration (Figure 8) and quantification is based upon comparison to an external standard curve. The radial immunodiffusion plates contain specific antiserum in agarose gel, 0.1 M phosphate buffer pH 7.0, 0.1% sodium azide as bacteriostatic agent, and 1 µg/mL amphotericin B as antifungal agent. Plates contain 0.002 Mehtylenediaminetetracetic acid. After filled with 5 microliters/well of the pure serum samples, the plates are incubated for 24 h at room temperature.

Total Protein Determination Total protein concentration in the serum samples was determined using the BCA Protein Assay (Smith et al., 1985). This is a detergent-compatible formulation based on bicinchoninic acid (BCA) for the colorimetric detection and quantification of total protein. This method combines the well-known reduction of Cu^{+2} to Cu^{+1} by protein in an alkaline medium (the biuret reaction) with the highly sensitive and selective colorimetric detection of the cuprous cation (Cu^{+1}) using an unique reagent containing bicinchoninic acid. The purple-colored reaction product of this assay is formed by the chelation of two molecules of BCA with one cuprous iron. This water soluble complex exhibits a strong absorbance at 570 nm that is nearly linear with increasing protein concentration over a broad working range (20-2,000 µg/mL). The BCA is not a true end point method; that is, the final color continues to develop. However, following incubation, the rate of continued color development is sufficiently slow to allow large numbers of samples to be assayed together. Protein concentrations were determined and reported with reference to Bovine Serum Albumin (BSA). A series of dilutions of known concentrations were prepared from the protein and assayed alongside the unknowns before the concentration of each unknown was determined based on the standard curve. Once the right dilution was

determined, the samples were loaded into the wells, incubated for 1 hour and read at 570 nm in a Synergy HT plate reader using KC4^{TM} v3.4 and KC4^{TM} signature software (Bio-Tek Instruments, Inc.; Vermont, USA).

Statistical Analysis. The data were analyzed using the GLM and REG procedures of SAS in order to establish relationships among sow parity, sow IgG colostral concentrations, birth order, and piglet birth weight to piglet serum IgG and protein concentration. We used a weighed logistic regression analysis in order to determine the effects of pig IgG concentration, piglet birth order and birth weight on % of survival at weaning, with number of piglets per point being the weighting factor. Individual pig was used as the experimental unit. Statistical differences were declared when $P < 0.05$ and trends were noted when $0.05 < P < 0.1$.

RESULTS

Sow colostral IgG concentration (Figure 1) explained 6% of the variation observed in pig IgG concentration ($P < 0.0001$). Piglet birth order and sow parity (Figures 2 and 4) accounted for 4% and 3% respectively of the remaining variation observed in pig IgG concentration ($P < 0.0001$). However, birth weight had no detectable effect (data not shown) on pig IgG concentration. Sow parity had a significant ($P < 0.001$) effect on IgG concentration on sow's colostrum with parities two to eight having greater IgG concentration than parity one (Figure 3).

Pig IgG concentration was strongly correlated ($P < 0.0001$) with total serum protein concentration (Figure 5). Piglet birth weight had no detectable effect on piglet blood serum total protein (data not shown).

Pig IgG concentration had both a negative linear and quadratic effect (Figure 6) on % survival at weaning ($P < 0.05$). Pigs with 1,000 mg/dl IgG or less (n=24) had a 67% survival at weaning; whereas, 39% of the pigs (n=247) had IgG concentrations between 2250 to 2500 mg/dl with 91% survival at weaning.

Birth order had no detectable effect on % survival at weaning (data not shown). Pig birth weight had a linear effect ($P < 0.05$) on % survival at weaning (Figure 7). Pigs weighing 0.9 kg (n = 107) at birth had 68% survival rate, and those weighing 1.6 kg (n = 158; ~average birth weight) had an 89% survival.

Piglet birth weight had a significant effect ($P < 0.0001$) on final market weight (Figure 8) but pig IgG concentration and piglet birth order did not (data not shown).

DISCUSSION

The most significant finding of this study was that sow parity, sow colostral IgG concentration and birth order only explained 13% of the total variability found in pig IgG concentration. We hypothesized that these factors would have accounted for a greater percentage of the total variability found in pig IgG concentration. Another significant finding was that, contrary to what we would have expected, birth weight had no effect on pig IgG concentration. This lack of effect could be explained by the fact that we physically aided

each new born piglet to suckle from their dam. Piglet IgG concentration and birth weight had the greatest effect of any of the variables measured on survival percent at weaning.

We selected piglets with birth weight greater than 0.68 kg because very low birth weight pigs die to a much greater extent than their heavier littermates and this would have limited our blood sampling at 2 d of age. Indeed, data collected on ~6000 piglets from first though seventh parity (PIC Camborough genotype sows), with average piglet birth weight of 1.6 kg and overall mortality of 12.7% indicates that pigs weighing less than 0.68 kg have a 60% rate of pre-weaning mortality (Mike Johnston, personal communication).

Blasco et al., (1995) reported that 12% of newborn piglets die before weaning. Moreover, > 50% of the deaths occurs in the first 3 d after birth (Dyck and Swerstra, 1987; Blasco et al., 1995) with crushing accounting for 43% of deaths (USDA, 1991). Most causes of deaths are due to interactions between the piglet and its environment (Le Dividich and Herpin, 1994). Xu et al. (2000) attributed these deaths during lactation to the increasing susceptibility to pathogens due to the low immune-competence of the piglets at birth.

The obvious question is then: how heritable is piglet mortality or survival rate? Rothschild and Bildanel (1998) reported estimates of heritability of piglet mortality at the level of litter and as trait of the sow as low as 0.05. Cecchinato et al. (2008) reported estimates of heritability as low as 0.03. It is then safe to assume that the greatest factors associated with piglet mortality are crushing and starvation. The latter is a consequence of the highly competitive environment among the littermates. Milk production of the sow is potentially a limiting resource causing competition among piglets because the voluntary feed intake of piglets keeps increasing, whereas milk production of the sow reaches a rather

constant level after 8 to 10 d post-farrowing (Harrell et al., 1993). Another potential source of interaction is the establishment of the teat order. Piglets tend to position themselves in a specific order at the teats during lactation (McBride, 1963). During the first h after birth, piglets push and bite each other to capture a teat, but competition is reduced right after the teat order has been established (Hartstock and Graves, 1976).

We specifically chose immunoglobulin G as the measuring index of circulating maternal antibodies because it is the most abundant immunoglobulin transferred from the blood to the mammary gland in swine. In sow colostrum, all the IgG, most of the IgM and 40% of the IgA originate from the maternal blood (Bourne and Curtis, 1973). Our results indicate that the concentration of pig serum IgG is sow colostrum IgG, birth order, and parity dependent. Klobasa et al. (2004) investigated passive immunity in 603 neonatal piglets and found that it was affected by birth order, litter size and parity.

We attribute the significant effect of birth order on pig IgG concentration to the rapid changes in colostrum quality and quantity between onset of birth and last piglet being born in each litter. Our results show that the average farrowing time for gilts was 2.4 ± 1.1 h, parity 2 was 2.2 ± 0.9 h and for parity 3 or more was 2.1 ± 1.2 h which is consistent with the farrowing interval reported by others researchers (Friend et al., 1962; Jones, 1966; Bourne, 1969a; Randall, 1972a). Bourne (1969a) reported that six h post-birth, the protein and gamma globulins fraction of colostral whey proteins decrease to 50% of the pre-nursing values. It is then safe to conclude that early-born pigs have access to colostrum 50% more concentrated in total protein and gamma globulins. Harmon et al., (1976) and Harstock and Graves (1976) reported that late-born piglets had higher mortality than earlier-born littermates. This

knowledge of colostrum concentration has led to the development of a swine production practices called “split suckling” in which pigs are removed from their dam at birth in order to allow early- and late-born piglets to have equal opportunity to acquire high levels of colostral protein.

Klobasa and co-workers (2004) also reported that birth weight was only of borderline significance in determining piglet IgG concentration. This is also in agreement with our results because we did not detect any relationship between birth weight and pig serum IgG content. Machado-Neto and co-workers (1987) showed that lower IgG in colostrum of sows was correlated with lower serum IgG in piglets in the first 20 d postpartum.

The levels of pig serum IgG found in our study agree in general with those reported earlier (Karlsson, 1996; Porter and Hill, 1970; Curtis and Bourne, 1971). Thirty-nine percent of the 2-3 d old pigs (n=247) in our study had IgG concentrations between 2250 to 2500 mg/dl, which is a little higher than those reported by Machado-Neto et al. (1987) by d 2 (2470 mg/dl) and d 3 (1940 mg/dl). This might be attributed to the fact that we carefully dried the pigs immediately after birth. There is no reference of any birth intervention in the work conducted by Machado-Neto and co-workers. This observation is quite important to disclose because Blecha and Kelly (1981) reported that a single 2.5-h exposure to cold air (15 C) temperature at birth reduces the subsequent acquisition of colostral immunoglobulin.

We hypothesized that pig serum IgG is highly correlated with pre-weaning mortality. We found both a linear and quadratic response when correlating pig serum IgG and piglet survival at weaning. Logistic regression was shown to be an appropriate and useful technique for analysis of factors affecting piglet survival. The advantages of logistic

regression are that it can be applied to continuous as well as discrete predictor variables and the fitted regression coefficients are readily translated into the odds ratio, giving a mathematical quantification to observed differences (Cieslack et al., 1983). We found that pigs need to have an IgG content of 1,000 mg/dl or less in order to have a 67% chance of survival. Machado-Neto and others (1987) found that a concentration of IgG of less than 10 mg/ml on postnatal d 1 has been associated with an increased pre-weaning mortality in piglets. We cautioned that the minimum level of serum immunoglobulin that should be achieved to ensure survival of piglets may depend upon the environment, farm management, seasons and disease conditions. Blecha and Kelly (1981) reported that live born piglets that die before 21 days of age had lower gamma globulin concentrations in serum during the first day of life than did pigs that live. These results are consistent with those reported by Hendrix et al. (1978) indicating that piglets that survived to 21 days of age had a higher concentration of gamma globulin, shorter birth interval, heavier birth weight and were born earlier in the litter than those pigs that were born alive but died before 21 days of age. Pigs from litters with high mortality rates show weight loss, do not synchronize in suckling, fight more and for longer periods and have lower IgG levels, indicating problems in the sow (de Passille et al., 1988; de Passille and Rushen, 1989b).

We argue that while systemic IgG concentration might indicate an animals' general immune status, circulating immunoglobulin content gives no indication as to the specificity of the immunity that may be present or that may have developed in the tissue immune system (e.g. IgA secreted in the intestinal tract), which is important in resisting certain diseases which are common in young pigs. We recognized that colostrum contains others substances

(i.e. energy) besides immunoglobulins that are important for piglet survival. Finally, a reduced consumption of immunoglobulins may predispose piglets to selected kinds of infectious diseases.

The reason we placed 11 pigs per sow was due to the interaction between the number of pigs in a litter and the stimulation they provide to sow's milk output. The fewer the pigs massaging, the longer the pre-ejection massaging needed to cause a milk ejection (Algers et al., 1990). The weight of the pigs at birth plays an important role in stimulating the sow to produce milk. A large pig may perform the massage of its teat before ejection more vigorously, thus achieving a greater blood flow to the teat and thereby bringing more of the limited supply of oxytocin to its own teat (Fraser, 1984b). It is well known that suckling leads to an activation of neurohormonal reflexes that result in the release of oxytocin (Folley and Knaggs, 1966), prolactin (van Landeghem and van de Weil, 1978), gut hormones such as gastrin, somatotropin and vasoactive intestinal polypeptide (VIP) and pancreatic hormones such as insulin and glucagon (Linden et al., 1987; Uvnas-Moberg and Ericksson, 1983; Uvnas-Moberg eat al., 1984; Algers et al., 1991).

Birth weight has been widely known to be a very important economic trait in swine production. Our results showing that low birth weight in piglets correlates with decrease survival and lower post-natal growth rates are consistent the work reported by others (Pond et al., 1985, Pond and Mersmann, 1998; Milligan et al., 2002; Quiniou et al., 2002) . Fix and See (2009) found that low birth weight piglets grew slower, were fatter and were more likely to die at weaning. Beaulieu and co-workers (2010) found that lighter birth weight pigs had reduced BW at weaning, 5 and 7 wk post-weaning, and at first pull and had increased days to

market. Rehfeldt and Kuhn (2006) argue that in the majority of low birth weight piglets low numbers of muscle fibers differentiate during prenatal myogenesis, for genetic or maternal reasons, and those low birth weight piglets with reduced fiber numbers are unable to exhibit postnatal catch-up growth. Prenatal development is mainly dependent on a close interrelation between nutritional supply/use and regulation by hormones and growth factors. They found that low birth weight piglets showed the lowest growth performance and the lowest lean percentage at slaughter. Wolter and co-workers (2002) investigated the effect of piglet birth weight and liquid milk replacer supplementation of piglets during lactation on growth performance to slaughter weight. The experiment included a 2 x 2 factorial arrangement of treatments were birth weight (heavy (1.8 kg) vs. light (1.3 kg BW) and supplemented vs. non-supplemented) were examined. Heavy pigs were heavier at weaning (6.6 vs. 5.7 kg BW), tended to have more pigs weaned (11.4 vs. 10.9 pigs/litter), had greater ADG (851 vs. 796 g/d) and required seven fewer days to reach slaughter weight when compared to their light counterparts. Feeding supplemental milk replacer during lactation produced heavier pigs at weaning (6.6 vs. 5.7 kg BW), tended to increase the number of pigs weaned (11.4 vs. 10.9 pigs/litter), required thee fewer days to reach 110 kg BW; but had no effect on growth performance from weaning to slaughter. They concluded that birth weight has a substantially greater impact on pig growth performance after weaning than increasing nutrient intake during lactation.

CONCLUSION

We found that sow colostrum, pig birth order and sow parity collectively had a small impact on pig IgG concentration and piglet birth weight did not. Piglet IgG concentration was highly correlated with piglet serum total protein concentration. Piglet IgG concentration and birth weight had the greatest effect of any of the variables measured on % survival at weaning. Piglet birth weight had a linear effect on final market weight. Collectively, these data indicate that pigs with ≤ 1000 mg/dl IgG at 2-3 days of age have reduced survivability and may benefit from IgG supplementation early in life. The number of pigs in this category was low in this study (24/637= 3.8%) but this number is biased downward because we excluded pigs weighing less than 0.68 kg from the experiment. If an IgG-rich supplement were directed to low birth weight pigs, pigs of high birth order and/or pigs from low-parity sows, survivability may be improved.

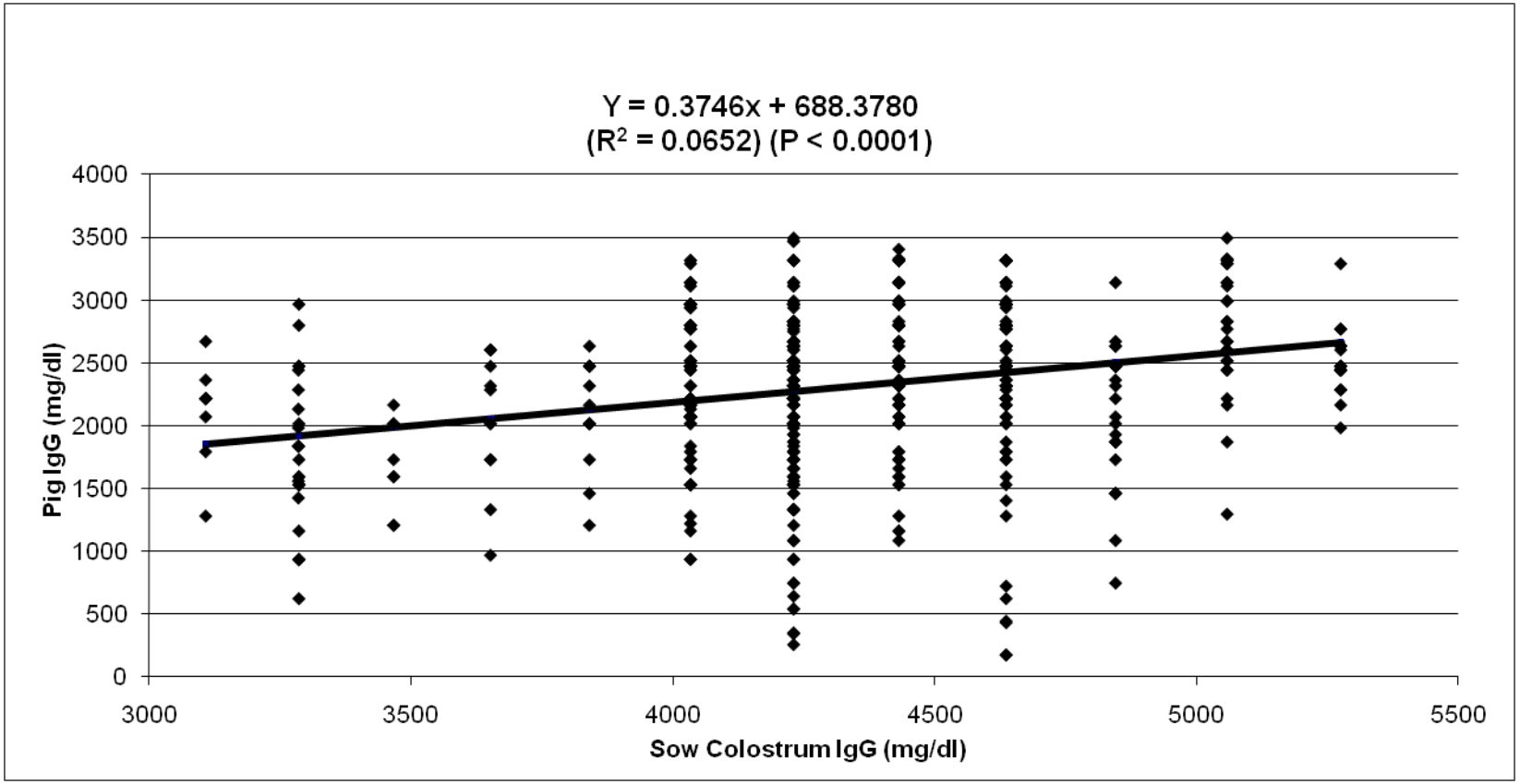

Figure 1. Effect of sow colostrum IgG concentration on pig IgG concentration.

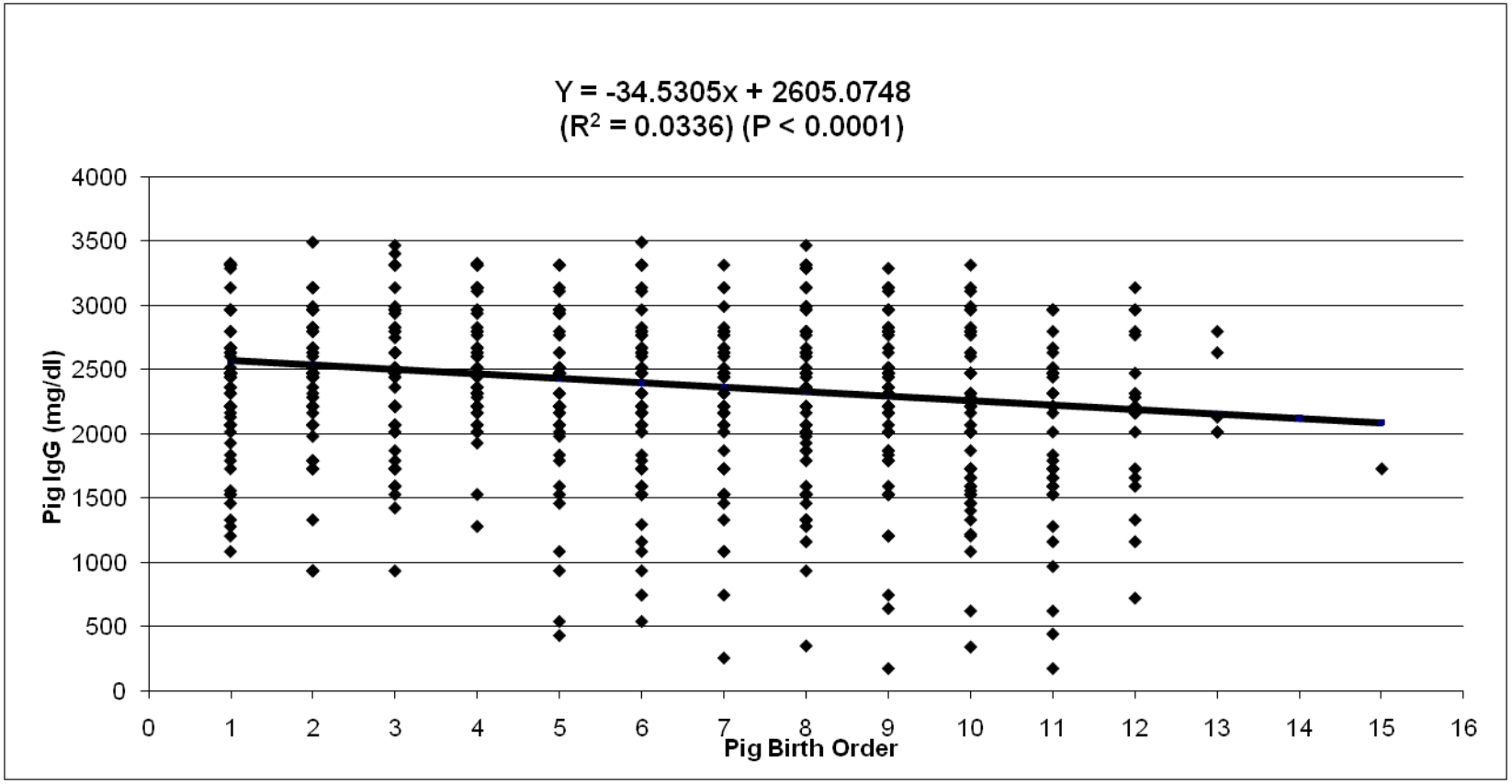

Figure 2. Effect of pig birth order on pig IgG concentration.

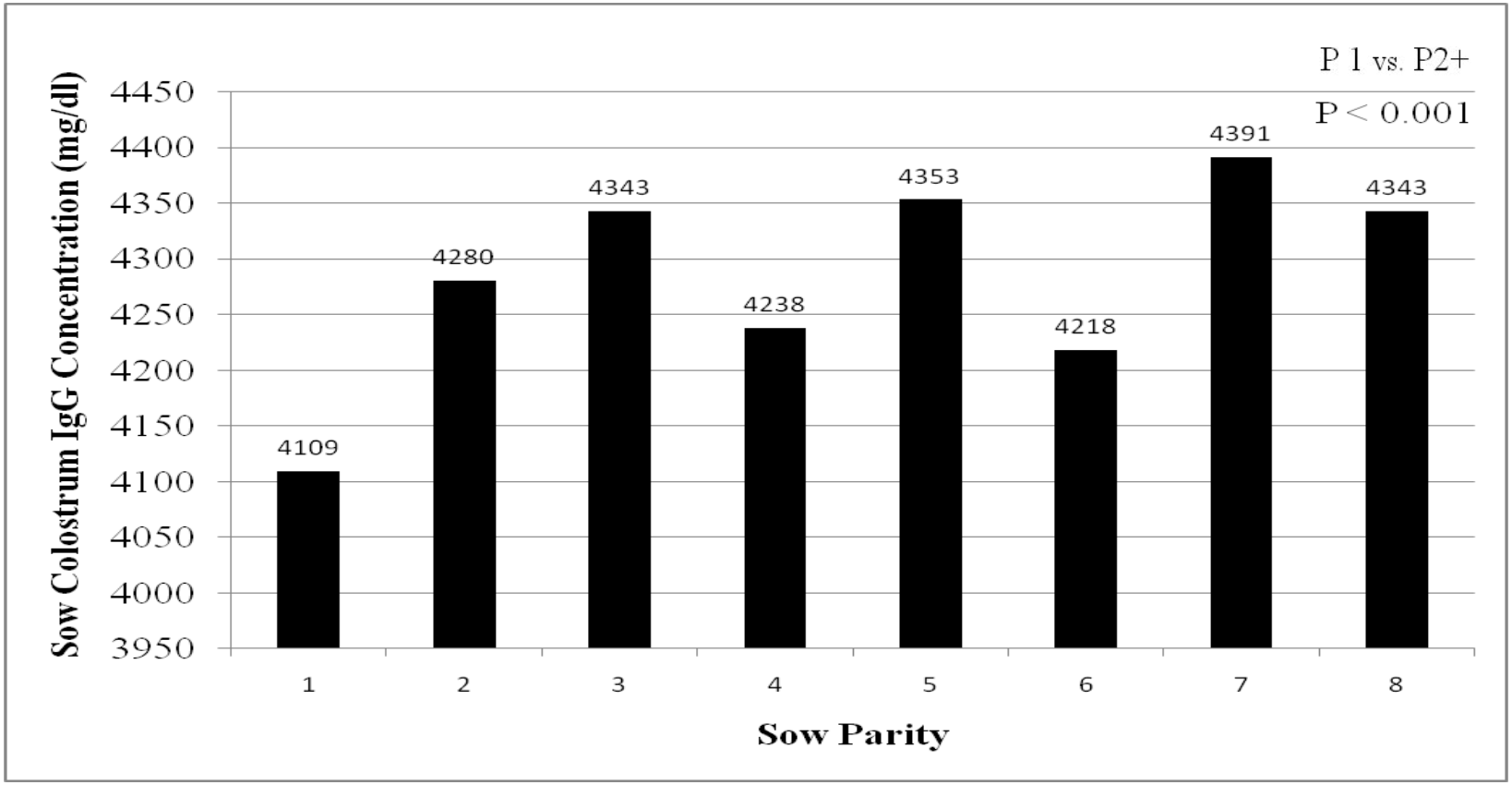

Figure 3. Effect of sow parity on sow colostral IgG concentration (mg/dl).

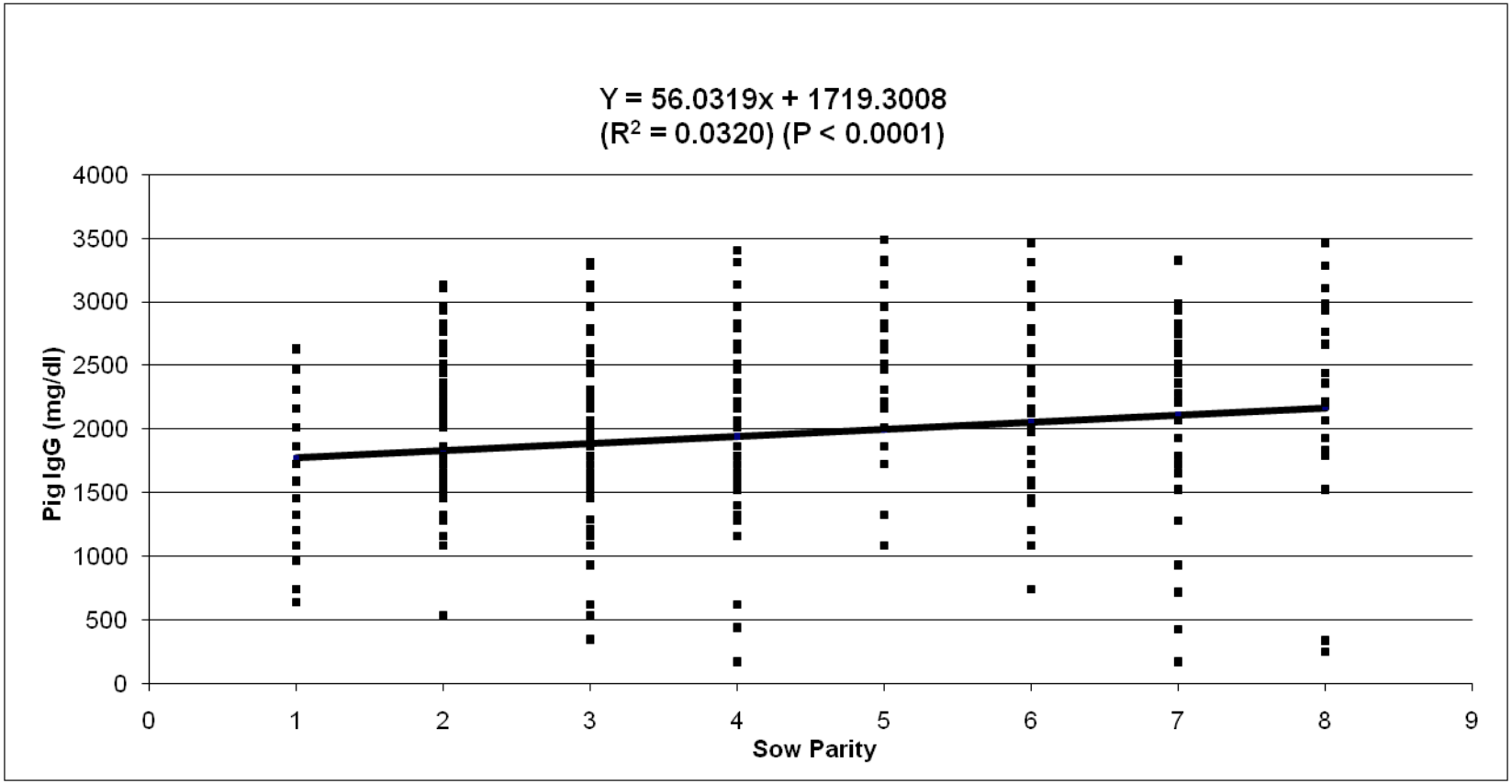

Figure 4. Effect of sow parity on pig IgG concentration.

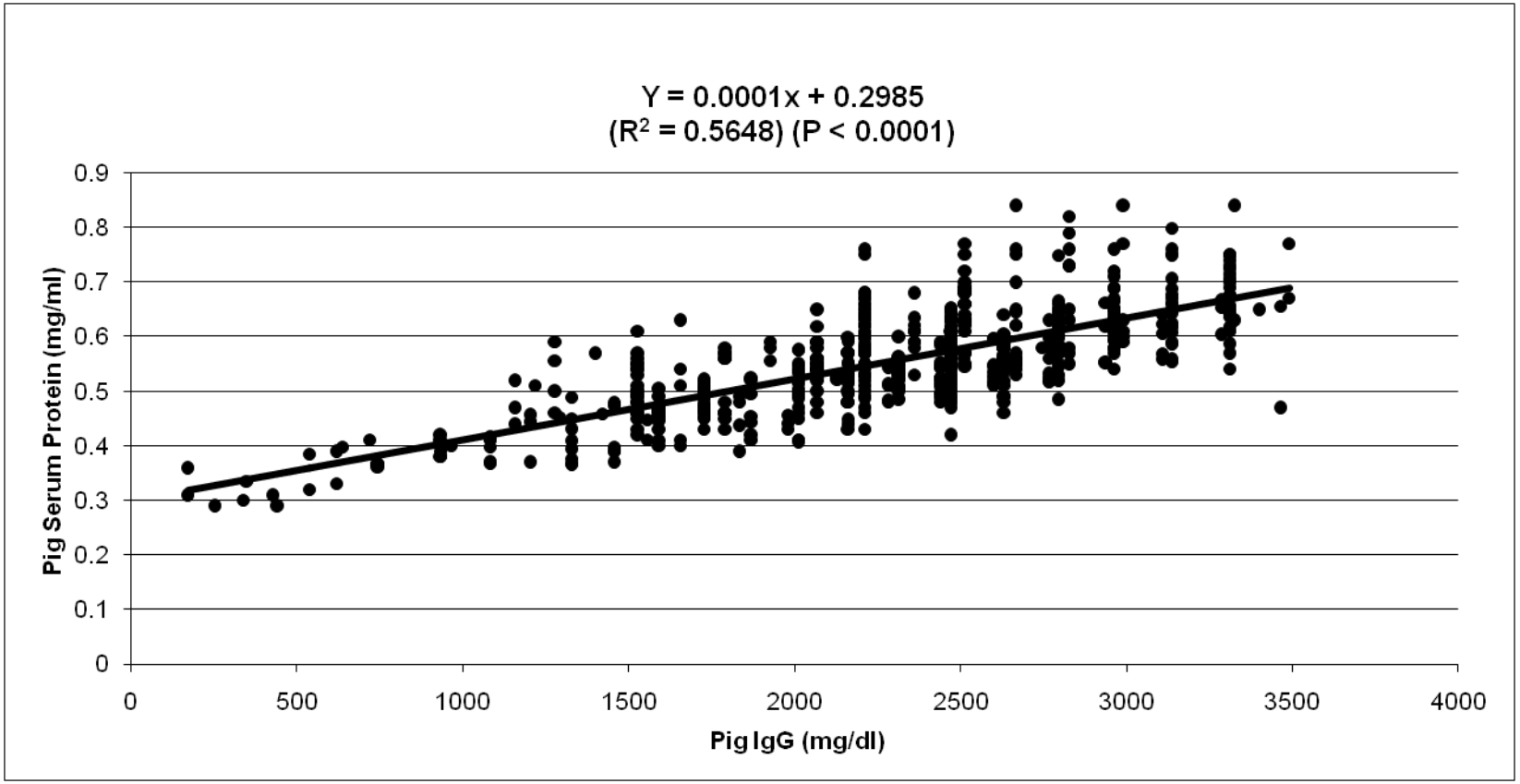

Figure 5. Effect of pig IgG concentration on pig serum protein concentration.

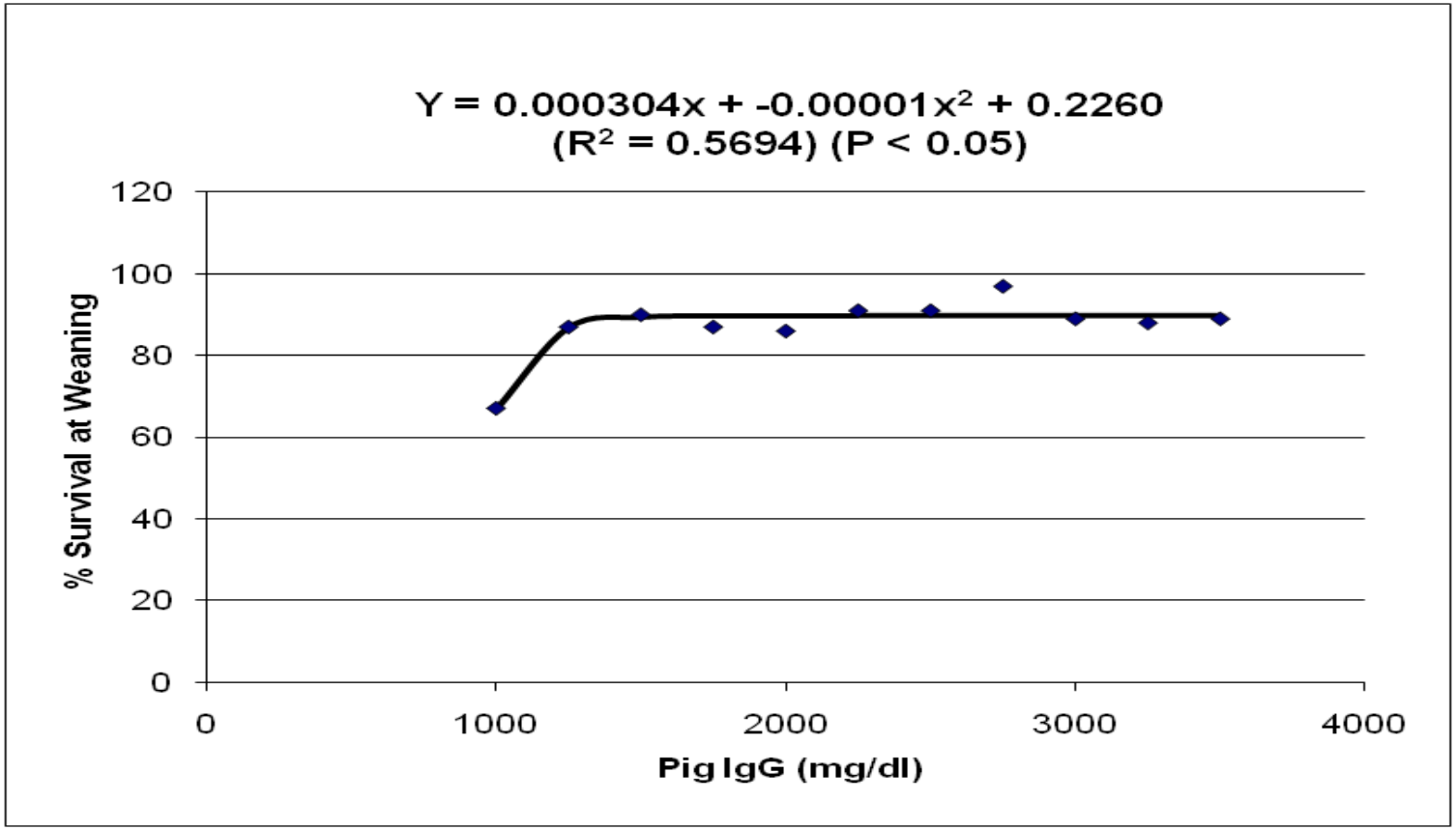

Figure 6. Effect of pig IgG concentration on % of survival at weaning.

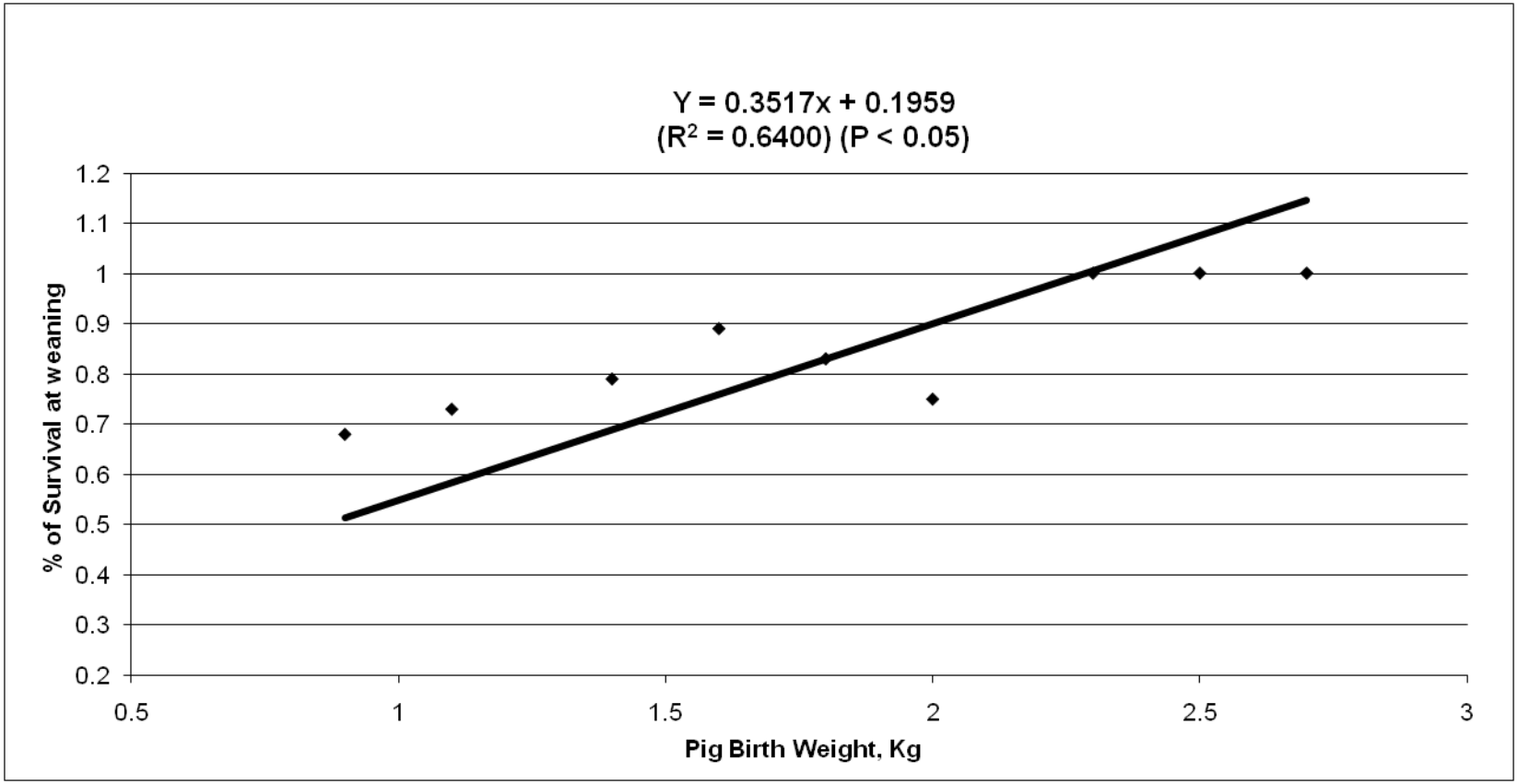

Figure 7. Effect of pig birth weight on % of survival at weaning

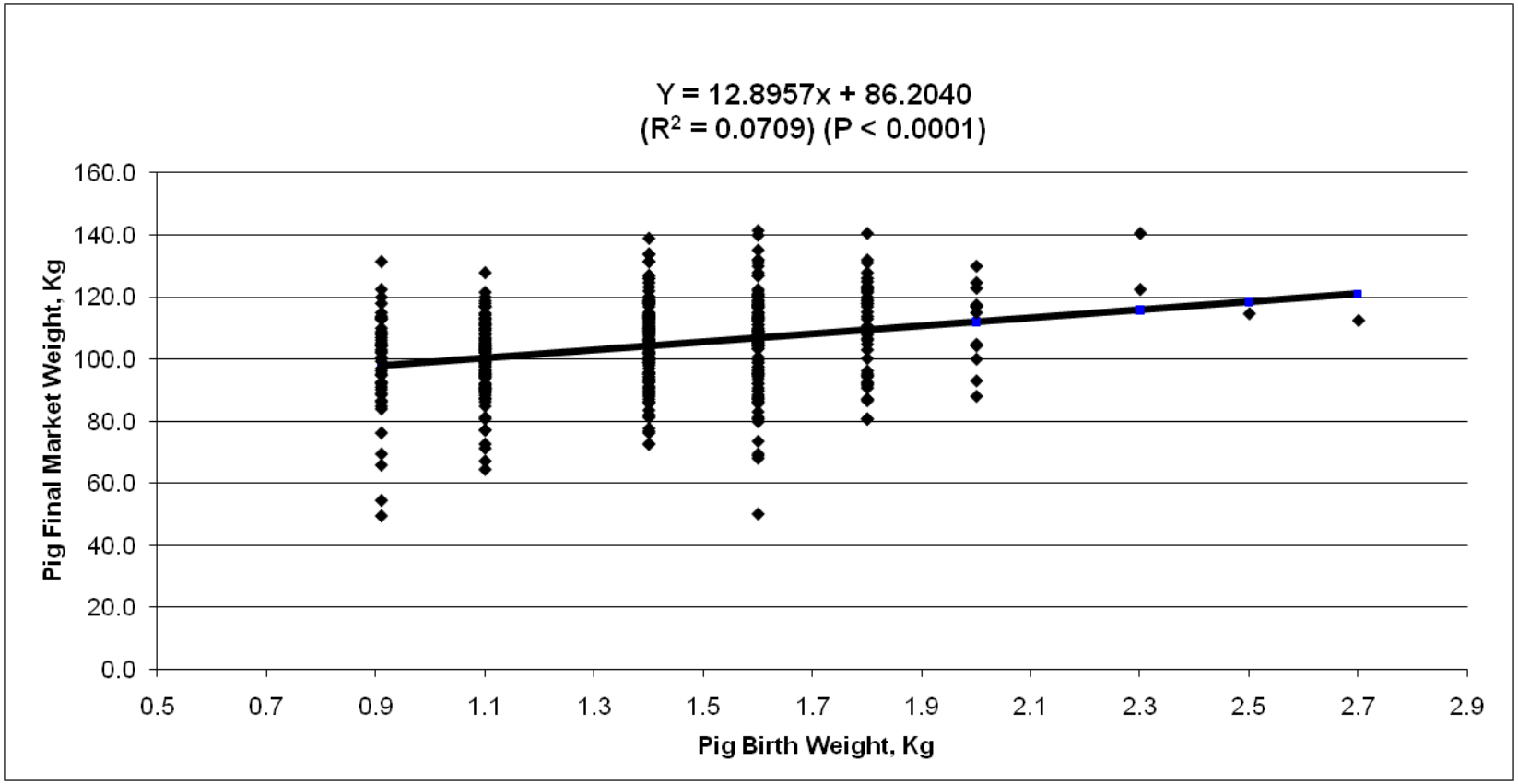

Figure 8. Effect of pig birth weight on pig final market weight.

Kent Laboratories, Bellingham, WA 98226

Figure 9. Radial Immuno Diffusion (RID) Technique.

LITERATURE CITED

1. Algers, G and P. Jensen. 1991. Teat stimulation and milk production in early lactation of sows. Can. J. Anim. Sci. 71:51.
2. Algers, B., S. Rojanasthein and K. Uvnas-Moberg. 1990. The relation between teat stimulation, oxytocin release and grunting rate in the sow. Appl. Anim. Behav. Sci. 26:267.
3. Blasco, A., J.P. Binadel and C.S. Haley. 1995. Genetics and neonatal survival. Pages 17-38 in The Neonatal Pig: Development and Survival. M.A. Varley, ed. CAB International., Leeds, UK.
4. Beaulieu, A.D., J.L. Aalhus, N.H. Williams and J.F. Patience. 2010. Impact of birth weight, birth order, and litter size on subsequent growth performance, carcass quality, muscle composition, and eating quality pork. J. Anim. Sci. 88:2767-2778.
5. Blecha, F and K.W. Kelly. 1981. Cold stress reduces the acquisition of colostral immunoglobulin in piglets. J. Ani. Sci. 52: 594-600.
6. Bourne, F.J. 1969a. Studies on colostral and milk whey proteins in the sow. 1. The transition of mammary secretion from colostrum to milk with natural suckling. Anim. Prod. 11:337.
7. Bourne, F.J and J. Curtis. 1973. The transfer of immunoglobulins IgG, IgA and IgM from serum to colostrum and milk in the sow. Immunology 24:157.
8. Cecchinato, A., V. Bonfatti, L. Gallo and P. Carnier. 2008. Survival analysis of preweaning piglet survival in a dry-cured ham-producing crossbred line. J. Anim. Sci. 86:2486-2495.

9. Cieslack, D.G., V.D. Leibbrant and N.J. Benevenga. 1983. Effect of a high fat supplement in late gestation and lactation on piglet survival and performance. J. Anim. Sci. 57:954-959.
10. Curtis, J and F.J. Bourne. 1971. Immunoglobulin quantification in sow serum colostrum and milk and the serum of young pigs. Biochim. Biophys. Acta 236:319.
11. De Passille, A.M. and J. Rushen. 1989b. Using early suckling behavior and weigh gain to identify piglets at risk. Can. J. Anim. Sci. 69:535.
12. De Passille, A.M., J. Rushen and G. Pelletier. 1988. Suckling behavior and serum immunoglobulin levels in neonatal piglets. Anim. Prod. 47:447.
13. Dyck, G.W. and E.E. Swierstra. 1987. Causes of piglet death from birth to weaning. Can. J. Anim. Sci. 68:543-547.
14. Fahey, J.L. and McKelvey, E.M. 1965. Quantitative determination of serum immunoglobulins in antibody agar plates. J. Immunol. 94: 84.
15. FASS. 1999. Guide for the care and use of Agricultural Animals in Agricultural Research and Teaching. 1st rev. ed. Fed. Anim. Sci. Soc., Champaign, IL.
16. Fix, J.S. and M.T. See. 2009. Piglet birth weight affects future growth, composition and mortality. North Carolina Swine Extension News, volume 32, number 2. College of Agriculture and Life Sciences. North Carolina State University, Raleigh, North Carolina.
17. Folley, S.J. and G.S. Knaggs. 1966. Milk-ejection activity (oxytocin) in the external jugular vein blood of the cow, goat and sow in relation to the stimulus of milking or suckling. J. Endocrinology 34:197.

18. Frazer, D. 1984b. The role of behavior in swine production. A review research. Appl. Anim. Ethol. 11:317.
19. Friend, D.W., H.M. Cunningham and J.W.G. Nicholson. 1962. The duration of farrowing in relation to reproductive performance of Yorkshire sows. Can. J. Comp. Med. 26:127.
20. Harmon, B.G., J.P. Totsch, D.J. Sprecher and P.J. Dziuk. 1972. Succession at birth and pig weight, growth and survival. J. Anim. Sci. 35:166. (abstract).
21. Harrell, R.J., M.J. Thomas, and R.D. Boyd. 1993. Limitations of the sow milk yield on baby pig growth. Pages 156-164 in Proc. 1993 Cornell Nutr. Conf. Feed Manufacturers. Cornell University, Ithaca, N.Y.
22. Hartsock, T.G. and H.B. Graves. 1976. Neonatal behavior and nutrition-related mortality in domestic swine. J.Anim. Sci. 42:235-241.
23. Hendrix, W.F. K.W. Kelley, C.T. Gaskins and D.J. Hinrichs. 1978. Porcine neonatal survival and serum gamma globulins. J. Anim. Sci. 47:1281-1286.
24. Jones, J.E.T. 1966. Observations on parturition on the sow. II. The parturient and post-parturient phases. Brit. Vet. J. 122:471.
25. Kalrsson, B.W. 1966. Immuno-electrophoretic studies on relationships between proteins of porcine colostrum, milk and blood serum. Acta Pathol. Microbiol. Scand. 67:83.
26. Klobasa, F., C. Schoder, C. Stroot, and M. Henning. 2004. Passive Immunization in neonatal piglets in natural rearing-effects of birth order, birth weight, litter size and parity. Tierarztl. Wochensch. 117:19-23.

27. Le Dividich, J. and P. Herpin. 1994. Effects of climatic conditions on the performance, metabolism and health status of weaned piglets: A review. Livest. Prod. Sci. 38:79-90.
28. Linden, A., M. Ericksson, M. Carlquist and K. Uvnas-Moberg. 1987. Plasma levels of gastrin, somatostatin and cholecystokinin immunoreactivity during pregnancy and lactation in dogs. Gastroenterol. 92:578.
29. Machado-Neto, R., C.N. Graves and S.E. Curtis. 1987. Immunoglobulins in piglets from sows heat-stressed prepartum. J. Anim. Sci. 65: 445-455.
30. Mancini, G., Carbonara, A.O. and Heremans, J.F. 1965. Immunochemical quantification of antigens by single radial immunodiffusion. Immunochemistry 2: 235.
31. McBride, G. 1963. The teat order and communication in young pigs. Anim. Behav. 11:53-56.
32. Milligan, B.N., D. Fraser and D.L Kramer. 2002. Within-litter birth weight variation in the domestic pig and its relation to pre-weaning survival, weigh gain and variation in weaning weights. Livest. Prod. Sci. 76:181-191.
33. PigCHAMP. 1998. PigCHAMP 1998 Datashare. PigCHAMP INC., Ames, Iowa. Available:http://www.pigchamp.com/1998Datashare.htm.
34. PigCHAMP. 2002. PigCHAMP 2002 Benchmarking. PigCHAMP INC., Ames, Iowa. Available:http://www.pigchamp.com/summary_archives.htm.
35. PigCHAMP. 2007. PigCHAMP 2007 Benchmarking. PigCHAMP INC., Ames, Iowa. Available:http://www.pigchamp.com/summary_archives.htm.

36. Pond, W.G., H.J. Mersmann and J.T. Yen. 1985. Severe feed restriction of pregnant swine and rats-effects on postweaning growth and body composition of progeny. J. Nutr. 115:179-189.
37. Pond, W.G. and H.J. Mersmann. 1988. Comparative response of lean or genetically obese swine and their progeny to severe feed restriction during gestation. J. Nutr. 18:1223-1231.
38. Porter, P. and I.R. Hill. 1970. Serological changes in immunoglobulins IgG, IgA and IgM and Escherichia antibodies in the young pig. Immunology 18:565.
39. Quiniou, N., J. Dargon and D. Gaudre. 2002. Variation of piglets birth weight and consequences on subsequent performance. Livest. Prod. Sci. 78:63-70.
40. Randall, G.C.B. 1972a. Observations on parturition in the sow. I. factors associated with the delivery of the piglets and their subsequent behavior. Vet. Rec. 90:178.
41. Rehfeldt, c and G. Kuhn. 2006. Consequences of birth weight for postnatal growth performance and carcass quality in pigs as related to myogenesis. J. Anim. Sci. 84:E113-E123.
42. Rothschild, M.F and J.P. Binadel. 1998. Biology and genetics of reproduction. Pages 313-343 in The genetics of the pig. M.F. Rothschild and A. Ruvinskty, ed.CAB International. Oxon, UK.
43. Smith, P.K., R.I. Krohn, G.T. Hermanson, A.K. Malia, F.H. Gartner, M.D. Provenzano, E.K. Fujimoto, N.M. Goeke, B.J Olson and D.C. Klenk. 1985. Measurement of protein using bicinchoninic acid. Analytical Biochemistry. 150:76-85.

44. USDA. 1991. National swine survey. Morbidity/mortality and health management of swine in U.S. national animal health monitoring system. Animal and plant health inspection service. Veterinary services. Washington, DC.
45. Uvnas-Moberg, K and M. Ericksson. 1983. Release of gastrin and insulin in response to suckling in lactating dogs. Acta Physiol. Scand. 119:181.
46. Uvnas-Moberg, K, M. Ericksson, L.E. Blomquist, A. Kunavongkrit, and S. Einarsson. 1984. Influence of suckling and feeding on insulin, gastrin, somatostatin and VIP levels in peripheral venous blood of lactating sows. Acta Physiol. Scand. 121:31.
47. Van Landeghem, A.A.J and D.F.M van de Wiel. 1978. Radioimmunoassay for porcine prolactin plasma levels during lactation, suckling and weaning and after TRH administration. Acta Endocrinol. 88:653.
48. Xu, R.J., F. Wang, and S.H. Zhang. 2000. Postnatal adaption of the gastrointestinal tract in neonatal pigs: A possible role of milk-borne growth factors. Livest. Prod. Sci. 66:95-107.
49. Wolter, B.F, M. Ellis, B.P. Corrigan and J.M. DeDecker. 2002. The effect of birth weight and feeding of supplemental milk replacer to piglets during lactation on pre-weaning and post-weaning growth performance and carcass characteristics. J. Anim. Sci. 80:301-308.

CHAPTER 4

EARLY POST-NATAL KINETICS OF COLOSTRAL IgG ABSORPTION IN FED AND FASTED PIGLETS AND DEVELOPMENTAL EXPRESSION OF THE INTESTINAL IgG RECEPTOR

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ABSTRACT

The transport of immunoglobulin G (IgG) across the epithelial barrier is achieved by the neonatal Fc receptor (FcRn) and this pathway provides passive immunity by delivering maternal immunoglobulin to the circulation of the neonate. The objective of this study was to determine the effect of time and feeding state on IgG absorption, intestinal morphology, and expression of IgG receptors in the first 24 h post-birth. Twenty newborn pigs were fitted with umbilical arterial catheters and gavaged with 32 ml defatted colostrum per kg of body weight either at birth (0 h) or at 12 h post-birth under either fed (milk replacer) or fasted (saline solution) conditions (n = 5/group). A fifth reference group (n=5), was euthanized at birth. Blood samples were drawn at 0, 1, 2, 4, 8 and 12 h post-gavage. At 12 h post-gavage, pigs were euthanized and jejunum tissues were collected for intestinal morphology and gene expression of FcRn and β_2 microglobulin **(**β_2m) via RTPCR. Pig serum IgG was determined by radial immunodiffusion. Data were analyzed according to a 2 X 2 factorial design (0 h-fed, 0 h-fasted, 12 h-fed and 12 hour fasted). There was no interaction between the time (age) of colostrum gavage (0 h vs. 12 h) and nutritional state (fed vs. fasted) for any of the parameters nor were there any differences between fed and fasted pigs. Serum IgG increased progressively with time, reaching peak concentration at 8 h post-gavage. Piglets given colostrum at 0 h had higher ($P < 0.05$) overall IgG absorption and higher ($P < 0.05$) villi height than those gavaged at 12 h post birth. Cycle thresholds of FcRn and β_2m were normalized to GAPDH. Abundance of FcRn transcript was lower ($P = 0.006$) in pigs euthanized at birth compared with those gavaged at 0 h and killed at 12 h of age. There was

no difference among the treatments in the expression of β_2m. In conclusion, the highest IgG absorption was realized 8 h after piglets were given defatted sow colostrum immediately at birth. Effects of delayed gavaging of colostral IgG and age-dependent changes in IgG receptor were modest over the first 24 h of life.

INTRODUCTION

Maternal antibodies play an eminent role in protecting neonatal piglets from infectious agents in the first few months of life, before they develop robust immunity of their own. Failure to passively transfer maternal antibodies can be devastating to the survival of the newborns (Ye et al., 2008). Neonatal piglets are born essentially agammaglobulinemic (Butler et al., 2002). Newborn piglets acquire IgG from colostrum by absorption though the intestine in the first 24 h to 36 h after birth, at which time the transport phase ceases (Lecce, 1973; Lecce et al., 1961; Hardy, 1969). The internalization of the macromolecules by enterocytes persists for an additional 2 to 3 weeks (Lecce, 1973; Clarke and Hardy, 1971; Leary and Lecce, 1976).

Recent studies reported by Schnulle and Hurley (2003) and Stirling et al. (2005) have shown that IgG is transported by the neonatal Fc receptor which is expressed in the mammary gland and intestine of the adult pigs. In addition to its role in transporting IgG, FcRn appears to protect IgG and albumin from being degraded by the lysosomes (Ghetie and Ward, 2000; Simister, 2003; Brambell et al., 1964; Roopenian et al., 2003; Brambell, 1966; and Chaudhury et al., 2003). Similar to the major histocompatability complex (MHC) class I and its related molecules, FcRn is composed of a heavy chain that is non-covalently attached

to a light chain β_2-microglobulin (β_2m) with a molecular weight of 12 kDa (Simister and Mostok, 1989; Burmeister et al., 1994). Apparently the presence of the β_2-microglobulin is essential for FcRn to exit the endoplasmic reticulum (Simister, 2003; Burmeister et al., 1994; Zhu et al., 2002).

Rodewalt (1973) and Jones (1976) argued that a completely specific and separate system exists (although no specific description is offered) for internalizing and transporting IgG to the circulation.

An important characteristic of the FcRn is that its interaction with IgG exhibits remarkable pH dependence. For example its binds IgG at acidic pH (6-6.5) and then releases IgG at neutral pH (7-7.4) (Ghetie and Ward, 2000; Rodewald, 1976; Zhu et al., 2005).

Quantitative Real-time RT–PCR is widely used, because of its high sensitivity, good reproducibility and wide quantification range (Bustin, 2000; Pfaffl and Hageleit, 2001). It is a sensitive method for detection and quantification of gene expression levels, particularly for low abundance mRNA (Bustin, 2000; Pfaffl and Hageleit, 2001), in tissues with low concentrations of mRNA (e.g. bone marrow, fatty tissues), from limited tissue samples (e.g. biopsies, single cells) (Lockey et al., 1998; Steuerwald et al., 1999) and to elucidate small changes in mRNA expression levels (Bustin, 2000; Pfaffl and Hageleit, 2001; Wittwer et al., 1997). However, it is a very complex technique with substantial problems associated with its true sensitivity, reproducibility and specificity and, as a fully quantitative methodology; it suffers from the problems inherent in real-time RT–PCR. Generally, two quantification strategies can be performed: an absolute or a relative quantification. In absolute quantification the absolute mRNA copy number per vial or capillary is determined by

comparison with appropriate external calibration curves (Pfaffl and Hageleit, 2001). An absolute quantification makes it easier to compare expression data between different days and laboratories, because the calibration curve is a non-changing solid and reliable basis. The relative expression is based on the expression ratio of a target gene versus a reference gene and is adequate for most purposes to investigate physiological changes in gene expression levels. We chose the relative expression method.

The objective of this experiment was to examine the effect of feeding de-fatted sow colostrum under fed and fasted conditions on the time course of IgG absorption, intestinal morphology and FcRn and β_2-microglobulin expression in the first 24 h post-birth.

MATERIALS AND METHODS

Colostrum Processing: The animal procedures in this research were approved by the North Carolina State University institutional animal care and use committee (IACUC). Samples of colostrum were manually collected from twelve lactating sows at the North Carolina State University Swine Educational Unit over a period of thee days (May 13, 14, and 15 of 2009). Between 40-80 mL (cc) of colostrum was collected in separate 120 mL (cc) sample cups from various sows within three different phases of parturition: prior to farrowing, during farrowing, and post farrowing. The samples were individually labeled with the sow identification number and parity of the sow. Individual parities of the sows ranged from one to seven. These colostrum samples were collected from P1 sows, eleven samples from P3 sows, eight samples from P4, four samples from P5 sows, and one sample from a P7 sow. Upon collection, the colostrum samples were stored at 4 ºC for further processing. In

the laboratory, 50 mL centrifuge tubes were filled with approximately 45 mL of the chilled colostrum samples in groups of ten. Tubes were centrifuged for 35 min x 1300 g (IEC Centra GP8R, DJB Labcare Company, UK) at 4°C to segregate the colostrum into two distinct layers: an upper layer composed of fat, and a protein rich lower layer which contained large amounts of IgG. The IgG-containing lower layer was collectively extracted from the tubes using a transfer pipette and pooled into a plastic storage container and stored frozen at – 20 °C. Ultimately, 840 mL of the IgG layer was extracted from the overall colostrum samples obtained.

Blood Collection and Processing: 20 colostrum-deprived pigs were catheterized immediately post farrowing. The umbilical catheters were polyurethane single-lumen catheters, measuring 3.5 French and 15" in length (Utah Medical Products, Inc.; Midvale, Utah). To insert the catheter into the portal vein, the cranial portion of the umbilical cord was cut about 5 cm from the pig's belly, exposing the lumen of the umbilical vein. The catheter was inserted about 10-20 cm so that the tip of the catheter was in the portal vein (Figure 1). During this procedure (adapted from Benevenga et al., 1992) the pig was sedated with isoflurane. A Jelco intermittent injection cap (Cardinal Health, Wright Cliff, KY) was attached to the end of the catheter in order to facilitate the bleeding process though a needle and syringe. The catheters were both sutured and taped to the skin. Heparinized saline (2,000 IU heparin /L physiological saline) was used to flush the catheters. The average time from birth until the first blood withdrawal was 15 minutes. Blood was collected every h for the first 2 h immediately after the catheter was inserted and then every 4 h until 12 h (0, 1, 2, 4, 8, and 12 h) for the treatments where the IgG portion was given right after birth. For the

treatments when the IgG was given at 12 h post birth, the sampling schedule was h 12, 13, 14, 16, 20 and 24 h. The collected blood samples were kept in ice until centrifugation at 1300 g for 25 minutes and the serum was then separated and frozen at – 20 C until IgG analysis.

Pig Handling and Feeding: Pigs were collected immediately after they were born without allowing suckling from their dams. They were dried off, weighed (Model S200 scale, Central City Scale; NE) ear tagged, and immediately catheterized. After catheter placement, pigs were allowed to recover under a heat lamp. The amount of IgG-rich portion was given at a dose of 32 mL/kg of BW. This amount was divided and gavaged in two doses, 60 min apart using oro-gastric feeding tubes measuring 41 cm x 3.3 mm (Kendall Dover™ Rob-nel ™, Tyco Healthcare Group LP, Mansfield, MS).

Piglets were gavaged according to a 2 x 2 factorial arrangement:

1) IgG gavaged at 0 h and then fed 20 mL of milk replacer (Advanced Birthright Nutrition, inc.; Delano, MN) at 2-h intervals until 12 h and then euthanized. The averaged birth weight for this treatment was 1.92 kg.
2) IgG gavaged at 0 h and then given 20 mL saline solution at 2-h intervals until 12 h and then euthanized. The averaged birth weight for this treatment was 1.6 kg.
3) IgG gavaged at 12 h and then fed 20 mL of milk replacer at 2-h intervals until they were euthanized at 24 h. Prior to the administration of the IgG-rich portion, they were fed milk at 2-h intervals starting at birth. The averaged birth weight for this treatment was 1.43 kg.
4) IgG-gavaged at 12 h and then given 20 mL saline solution at 2-h intervals until they were euthanized at 24 h. Prior to the administration of the IgG-rich portion,

they were fed saline solution at 2-h intervals starting at birth. The averaged birth weight for this treatment was 1.67 kg.

Immunoglobulin G Determination: We followed the Radial Immunodiffusion test (RID) described in Chapter 3.

Efficiency of Absorption: The efficiency of absorption of IgG was calculated by dividing the total calculated amount of IgG in piglets plasma by the amount supplied to each piglet x 100% as described by Bikker et al. (2010).

H & E Staining: Jejunum samples were collected (25 cm from the stomach) and preserved in a formalin solution and stored in room temperature for histology analysis. Tissues were trimmed into five mm thick sections and placed in processing cassettes. The tissues were processed in a Tissue-Tek VIP5 tissue processor (Sakura Finetek, Torrance, CA) using a standard overnight processing schedule. Tissues were embedded in paraffin and five micron sections were mounted on glass slides. The slides were stained on a DRS-601 slide stainer (Sakura Finetek, Torrance, CA) with hematoxylin and eosin, cleared and mounted with a permanent media. The staining method involves application of hemalum, which is a complex formed from aluminum ions and oxidized haematoxylin. This colors nuclei of cells (and a few other objects, such as keratohyalin granules) blue. The nuclear staining is followed by counterstaining with an aqueous or alcoholic solution of eosin Y, which colors eosinophilic structures in various shades of red, pink and orange (Figure 3). The stained tissues in glass slides were examined using an Olympus AH-2 Vanox-S microscope (Ultrasonic Power Corporation, Freeport, IL) and measured using SPOT^{TM} software (SPOT^{TM} Imaging Solutions, Sterling Heights, MI).

RNA extraction: We scraped the mucosa of the jejunum of 25 neonatal piglets (5 pigs at birth, 10 pigs at 12 h post-birth and 10 pigs at 24 h post-birth) and then froze it in liquid nitrogen and stored at -80 ^{0}C until RNA extraction was performed. Total RNA from the pigs' jejunum mucosa was extracted using RNeasy kit (Qiagen, Germantown, MD). The total RNA was quantified using a NanoDrop Spectrophotometer 2000C (Thermo Scientific, Wilmington, DE) and the quality was evaluated by 1.2% agarose gel electrophoresis. Intact 28S and 18S RNA were observed on the gel indicating minimal or no degradation of the RNA (Figure 2).

cDNA Synthesis: One µg of total RNA was used to synthesize cDNA using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Inc., Foster City, CA, USA) according to manufacturing recommendations. Prior to use, cDNA was diluted 1:20 with H_2O.

Real Time PCR: Primers for the genes FcRn (neonatal Fc Receptor), β_2m (beta-2-microglobulin) and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) were designed using the software program Beacon Designer^{TM} (Premier Biosoft International, Palo Alto, Ca) (Table 1). GAPDH was selected as the housekeeping gene due to its expression stability in different pig tissues (Garcia-Crepo et al., 2005; Foss et al., 1998). The optimum annealing temperatures were 57 ^{0}C for FcRn, 58 ^{0}C for β_2M and 58 ^{0}C for GAPDH. Single reactions were prepared for each cDNA along with each serial of dilution using the ABI SYBR Green Master Mix (Applied Biosystems, Inc., Foster City, CA, USA). A negative control (no reverse transcriptase) was included in order to confirm the absence of genomic DNA. Each

PCR reaction consisted of 20 µL containing 1 µL of 1:20 diluted cDNA, 0.4 µL of forward primer, 0.4 µL of reverse primer, 0.2 µL of Fluorocein, 10 µL of ABI SYBR Green and 8 µL of DEPC (Diethylpyrocarbonate) water (Fisher Scientific, Pittsburg, PA). The real time qPCR was run on Bio-Rad iCycler iQ^{Tm} (Bio-Rad Laboratories, Hercules, CA). The cycling program was as reported by Hansen et al. (2009).

***Comparative Cycle Threshold (C_t) Method**:* Real-time RT-PCR is as powerful tool to quantify gene expression. The quantitative endpoint for real-time PCR is the threshold cycle (C_T). The C_T is defined as the PCR cycle at which the fluorescent signal of the reporter dye crosses an arbitrarily placed threshold (Schmitten and Livak, 2008). The numerical value of the C_T is inversely related to the amount of amplicon in the reaction (i.e., the lower the C_T, the greater the amount of amplicon. We compared the change in C_T values (ΔC_t) of two treatments (0 h and 12 h) vs. control (Birth) in order to determine the relative changes in expression for the FcRn and β_2M genes. Again they were both normalized to GAPDH. The comparative C_T method makes several assumptions, including that the efficiency of the PCR is close to 1 and the PCR efficiency of the target gene is similar to the internal control gene (Livak and Schmittgen, 2001).

STATISTICAL ANALYSIS: Total IgG absorption and tissue histology were analyzed using the PROC GLM procedures of SAS. For the gene expression analysis, we used the Relative Expression Software Tool (REST©) developed by Pfaffl and others (2002). The reason we chose REST© is because all published equations and available models for the calculation of relative expression ratio allow only for the determination of a single

transcription difference between one control and one sample. REST© compares two groups, with up to 16 data points in a sample and 16 in a control group, for reference and up to four target genes. The mathematical model used is based on the PCR efficiencies and the mean crossing point deviation between the sample and control group. Subsequently, the expression ratio results of the four investigated transcripts were tested for significance by a randomization test.

Pig was the experimental unit. Birth weight was used as covariate. LS means were calculated and $P < 0.05$ was chosen to determine their significance.

RESULTS

We found no interaction ($P > 0.05$) between time of colostrum gavage and the status of feeding (fed or fasted). However, pigs gavaged with colostrum at 0 h (right after birth) had 264 mg/dL (or 25.7%) higher ($P < 0.05$) IgG concentration than those given the defatted colostrum 12 h post-birth (1290 vs. 1026 mg/dl respectively; Figure 4). It took 2 h to detect any IgG concentration for those pigs fed at 0 h and 4 h for those gavaged at 12 h post birth. The efficiency of IgG transport in pigs gavaged at 0 h of age exceeded ($P < 0.05$) the efficiency of pigs gavaged at 12 h by 4.4% (21.5 vs. 17.1%; Figure 6).

Consistent with the reported results above, the villi of pigs at 12 h of age were 20% higher ($P < 0.05$) than the pigs at 24 h of age (876 vs. 728 microns respectively; Figure 7). There were no significant differences ($P > 0.05$) between the two treatments in villi width and crypt depth.

The gene expression of FcRn of the pigs at 12 h of age was up-regulated by 1.8 fold ($P = 0.006$) over piglets at birth (Figure 8). In contrast, FcRn expression in 24-h-old pigs was not different ($P = 0.928$) than newborn pigs. There was no difference among treatments in the expression of β_2 microglobulin (data not shown).

DISCUSSION

Colostrum itself plays a most important role in the induction of intestinal closure. The formation of colostrum in the sow starts about one month prepartum with an intensive transfer of immunoglobulins from the serum to the udder (Jonsson, 1973). This process virtually ceases after parturition resulting in a sharp decrease of the IgG concentration in the colostrum (Frenyo, 1980; Klobasa, 1986). These authors also confirmed that IgG absorption by the piglet starts declining 24 h postpartum (Figure 3). Blecha (1998) indicated that the IgG concentration of colostrum is highest during the birth process and decreases during the first day of lactation.

Butler et al. (2002) reported that low amounts of maternal IgG may pass the placenta though the fetus. Le Dividich et al. (2005) found that immunoglobulin levels transfer via the placenta are negligible. We were unable to detect any IgG which may have passed from the placenta to the fetus immediately after birth (Figure 4). These findings confirm that neonatal piglets at birth possess almost no serum immunoglobulins and undetectable levels of specific antibodies. It took more than one h to detect any IgG in piglet serum for animals gavaged at birth and more than 2 h for the pigs gavaged at 12 h of age, regardless of whether they were

fed or fasted. Werhahn et al. (1981) reported that labeled porcine IgG was found in the blood of artificially reared piglets as early as 4 h after administration.

The efficiency of porcine IgG transported to the blood in our study did not differ between fed and fasted animals. This is consistent with data reported by Werhahn et al. (1981). They reported that piglets starved for 24 h had absorbed no less IgG than those given IgG the first 2-4 h after birth. They also reported that absorption ability was preserved in piglets starved for 106 h after birth, merely given water three times a day.

The offering of the porcine IgG right after birth (0 H treatment) versus waiting 12 h (12 H treatment) yielded 4.4% more IgG transported to the blood (21.5% vs. 17.1%) (Figure 6) perhaps due to the significant ($P < 0.05$) larger villi found in their jejunum of 0 H treatment pigs (Figure 7). This explains the difference reported in the % of IgG transported from the gut to the blood stream found between the two treatments. We assume that the larger the villi the greater the absorption of nutrients in this case the IgG present in the defatted colostrum. These results are consistent with those reported by Leary and Lecce (1979). They gavaged piglets with 500 mg of porcine IgG plus 500 mg of bovine albumin and they observed a 21.7% increase in the IgG transported to the blood. They also gavaged piglets with 500 mg of porcine IgG plus 500 mg of porcine albumin and observed a 17.6% increase in the IgG transported to the blood. By some unknown mechanism, bovine colostrum, when compared to mature milk, is able to augment the absorption of porcine IgG (Werhahn et al., 1981). Bikker et al. (2010) removed piglets from dam prior to suckling and allocated them to five treatments. Piglets received either 28 g of standardized sows colostrum per kg of BW (control) or one out of four products containing porcine IgG and fats, porcine

IgG and glycerine, porcine IgG and dextrose and fat and last porcine IgG and dextrose and dried fat. After 12 h of fasting during which only water was given to all groups, they recorded the efficiency of IgG absorption to be 19.5%, 22.8%, 14.5%, 27.7% and 4.8% respectively for all groups. They concluded that the absorption of IgG from a single dosed oral supplement of a porcine plasma derived product can be as high or higher that IgG absorption from a single dose of sow colostrum.

Newborns piglets acquire IgG from colostrum by absorbing though the intestine over a period of nearly 36 h postpartum. We argue that this time may be shorter than we anticipated (Figure 8). Our data indicates that there is a down-regulation of the FcRn receptor 24 h postpartum. Epithelial cells of neonatal rodents possess specific IgG receptors which disappear in parallel to the waning of neonatal absorption (Guyer et al., 1976; Borthistle et al., 1977). Whether or not the gut could indefinitely retain the capacity to absorb IgG in the absence of ingested food remains questionable and unknown from our findings.

Various researchers (Staley et al., 1969; Kraehenbuhl and Campiche, 1969; Rodewald, 1973) have indicated that the lost absorptive capacity of the piglet's gut must not depend solely on a change in the gut epithelial cells. Kraehenbuhl and Campiche (1969) argued that closure may involve a change not in the uptake by gut cells of IgG but rather a change in the intracellular enzymatic activity of the gut cells. The other issue in question is to recognize whether there are differences in absorption among the different IgG subpopulations. We did not consider such differences in the design or the interpretation of our results.

Many researchers (Carlisle and Beck, 1983; Smith and Jarvis, 1978; Moon, 1971; Smith, 1985) have conducted trials to determine whether gut closure is a function of immature cells being replaced by mature cells in the small intestine. This effort seemed to be answered by Rundell and Lecce (1971) who concluded that intestinal epithelial cell turnover and closure are not related to one another in species other than the rat. They performed combined auto radiographic and fluorescent absorption marker studies on neonatal mice, hamsters, rabbits, guinea pigs and pigs. Their findings yielded two specific outcomes 1) the turnover time of porcine intestinal epithelium is about five days and 2) that closure (uptake and transport) occurred before the initial cell population was replaced.

Meanwhile, Clarke and Hardy (1971) argued that the process of incorporation of IgG into the circulation of neonatal pigs stops when the enterocytes are unable to discard the absorbed IgG though the lateral or basal membranes. Moon's (1971) work supported Lecce and Rundell's findings. He conducted an auto radiographic study injecting ^{3}H-thymidine into intestinal epithelium. He found that the jejunal-ileal epithelial cells were not extruded at two or three days of age according to research in rats, but the cells remained on the villi for seven to ten days, an age which is beyond the normal onset of closure. This conclusion is in agreement with the work of other researchers (Clark and Hardy, 1971; Lecce, 1973) who reported that the neonatal piglet could still take up but not absorb macromolecules for several days following closure.

Smith and Jarvis (1978) conducted similar study supporting Moon's findings. In a similar study using ^{3}H-thymidine, they found that labeled cells originated in the crypts and migrated onto the villi of neonatal piglets at a rate of 0.4 cells /hour. This rate has remained

unchanged and independent of the area of the small intestine throughout the first four days post-partum. They concluded that the cessation of uptake and the acquired resistance of piglets to viral insult were dependent on the final disappearance of cells which were present at birth in the neonatal pig intestinal epithelium.

The ages at which macromolecular uptake stops and the intestinal epithelium of the neonatal is replaced seem to be the same. However closure seems to occur independently of both mechanisms. We could then hypothesize that similar to the intestinal epithelial cells of the rats, the intestinal epithelial cells of the neonatal pig retain their capacity to uptake macromolecules throughout their lifespan but unlike the rat, they lose their ability to transport macromolecules to the circulation some time before they are shed from the villi. This time is before five days post-partum.

CONCLUSIONS

The highest IgG absorption was observed when piglets were given colostrum immediately after birth. Pigs supplemented at birth had higher villi height than those supplemented at 12 h. They also had a higher percentage of IgG transported in the blood when compared to those supplemented at 12 h. The relative FcRn expression increased transiently in 12-h-old when compared to the other treatments.

The results found in this study indicate that if there is an interest in supplementing a sow/bovine colostrum supplement to piglets to improve their immune status, the recommended time to gavage is immediately at birth.

Further studies are needed to examine the other mechanisms proposed that a specific and separate system exists for internalizing and transporting IgG to the circulation. Whether absorption of IgG by the neonatal piglet is receptor mediated or by a specific/separate system, the whole mechanism is reasonably complicated and highly regulated.

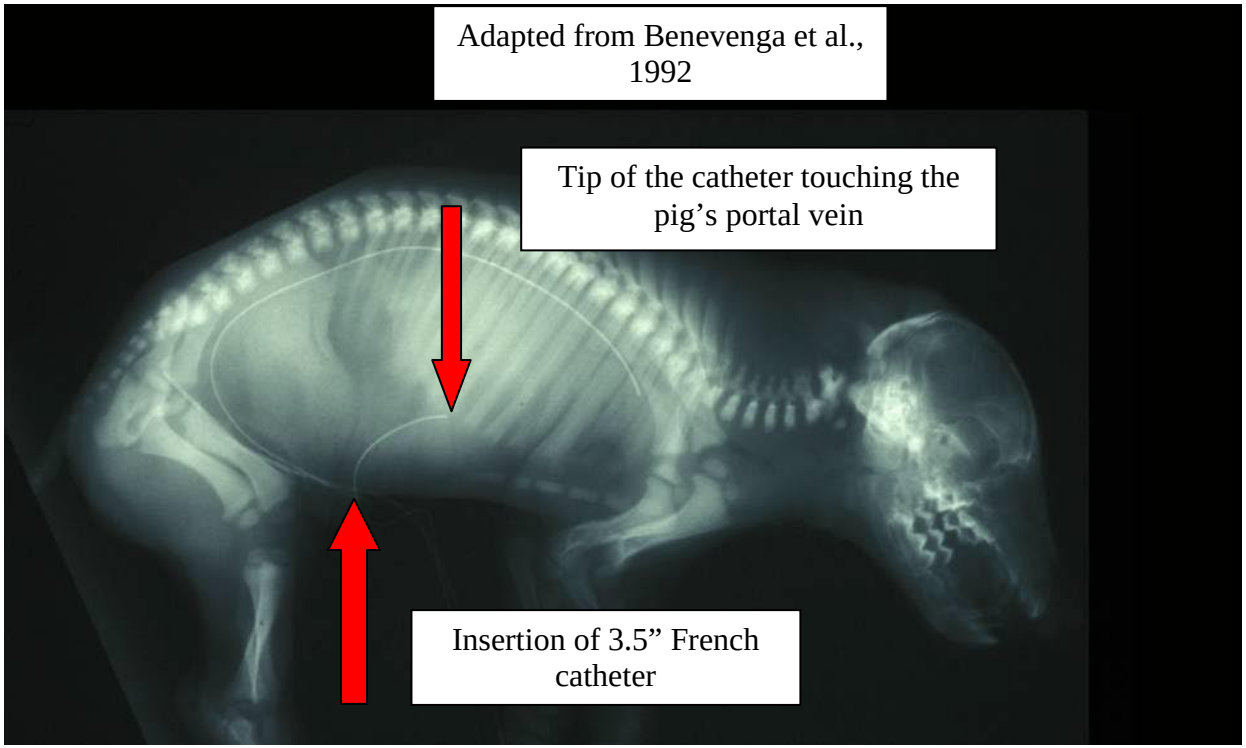

Figure 1. Insertion of the Single Lumen 3.5 French Catheter into the Pig's Portal Vein.

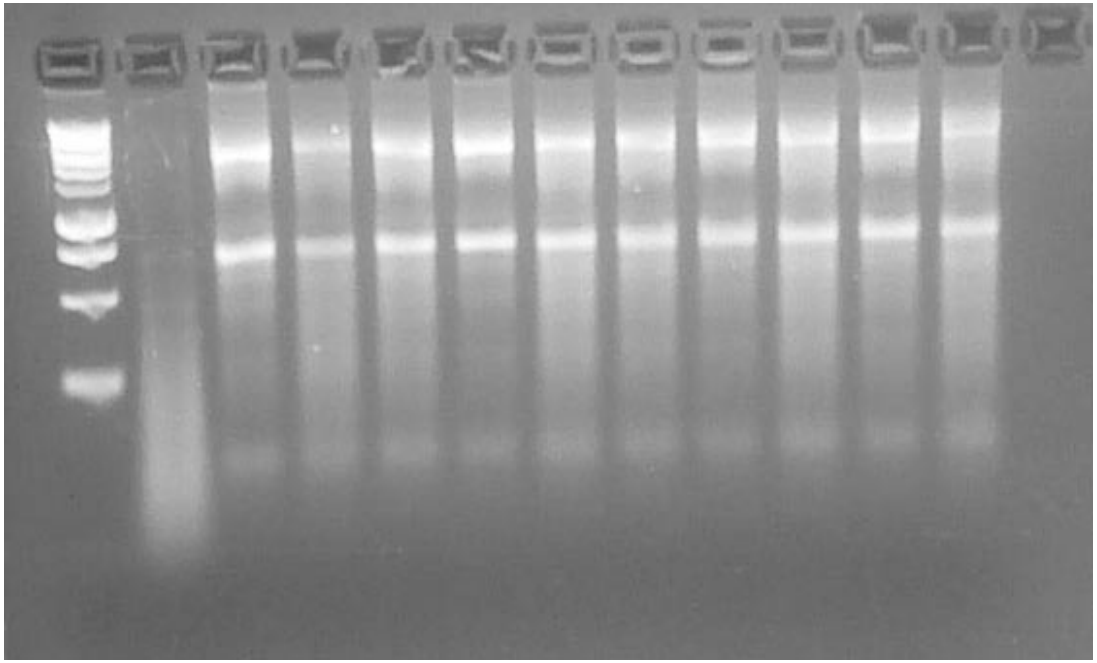

Figure 2. Quality of RNA from Pigs' Jejunum as Evaluated by 1.2% Agarose Gel Electrophoresis.

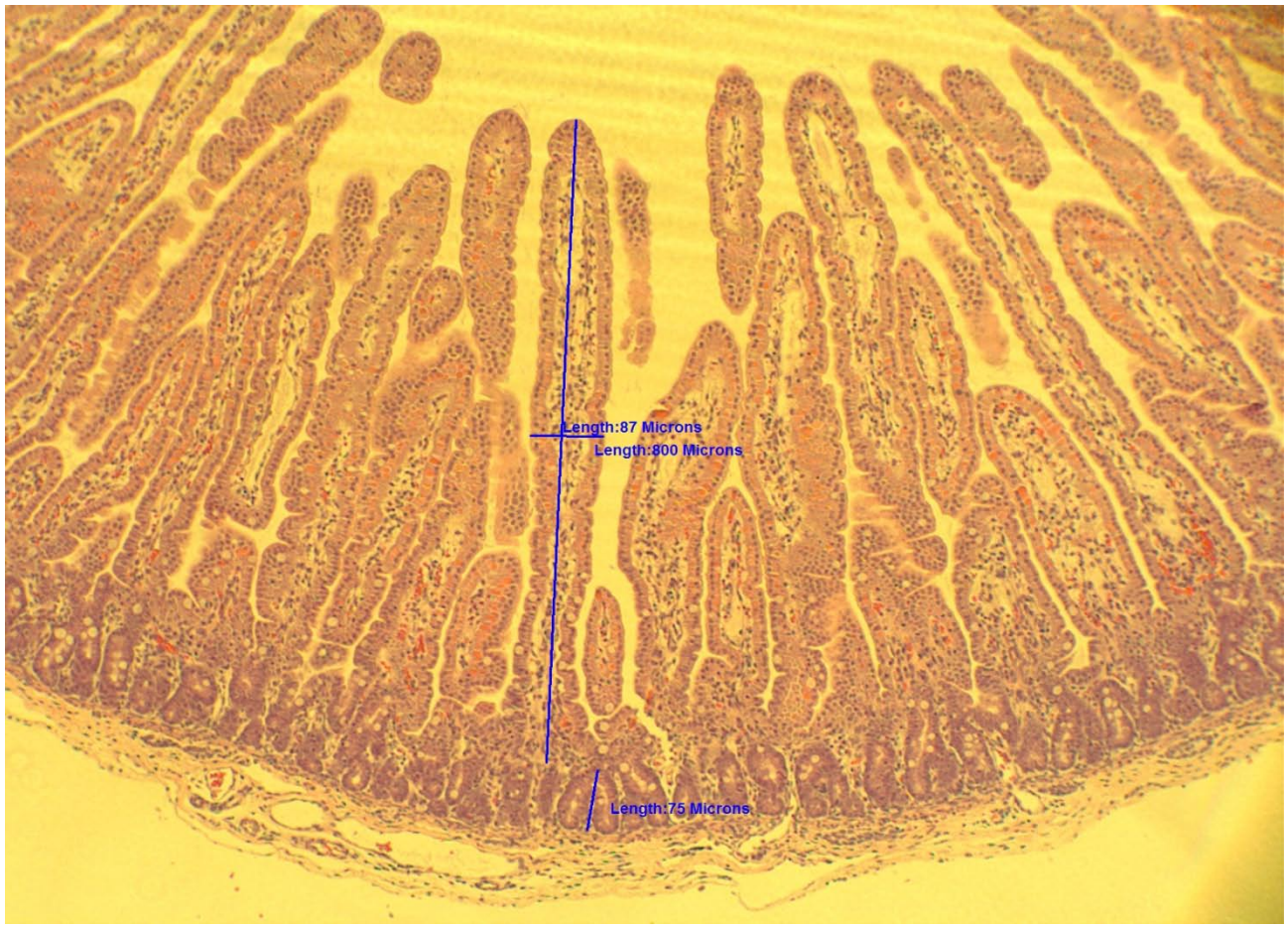

Figure 3. Micrograph of the Measurement of Villi Height, Width and Crypt Depth in a 1-Day-Old Piglet Jejunum.

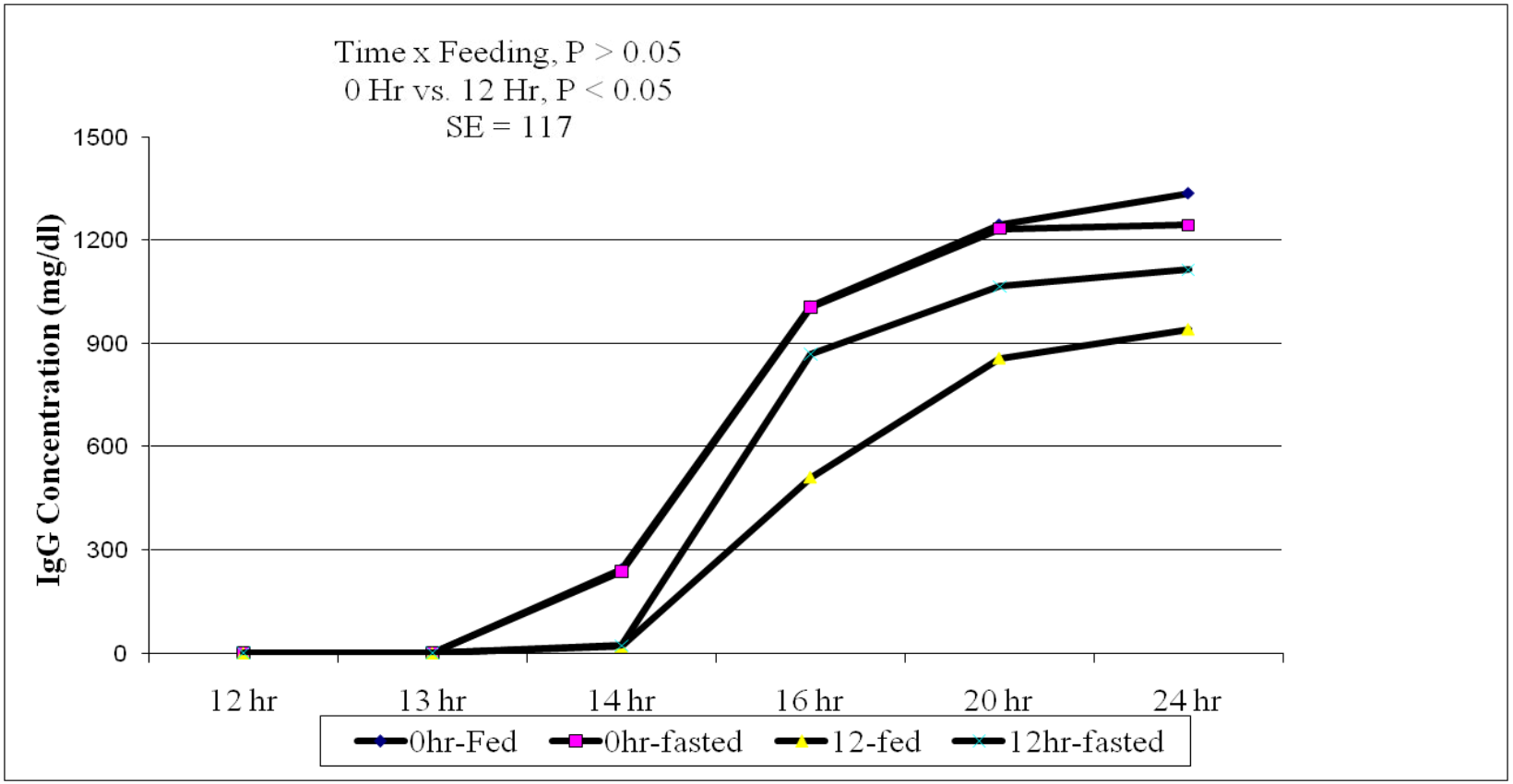

Figure 4. Time Course of IgG Absorption in Fed and Fasted Pigs Gavaged with Colostrum.

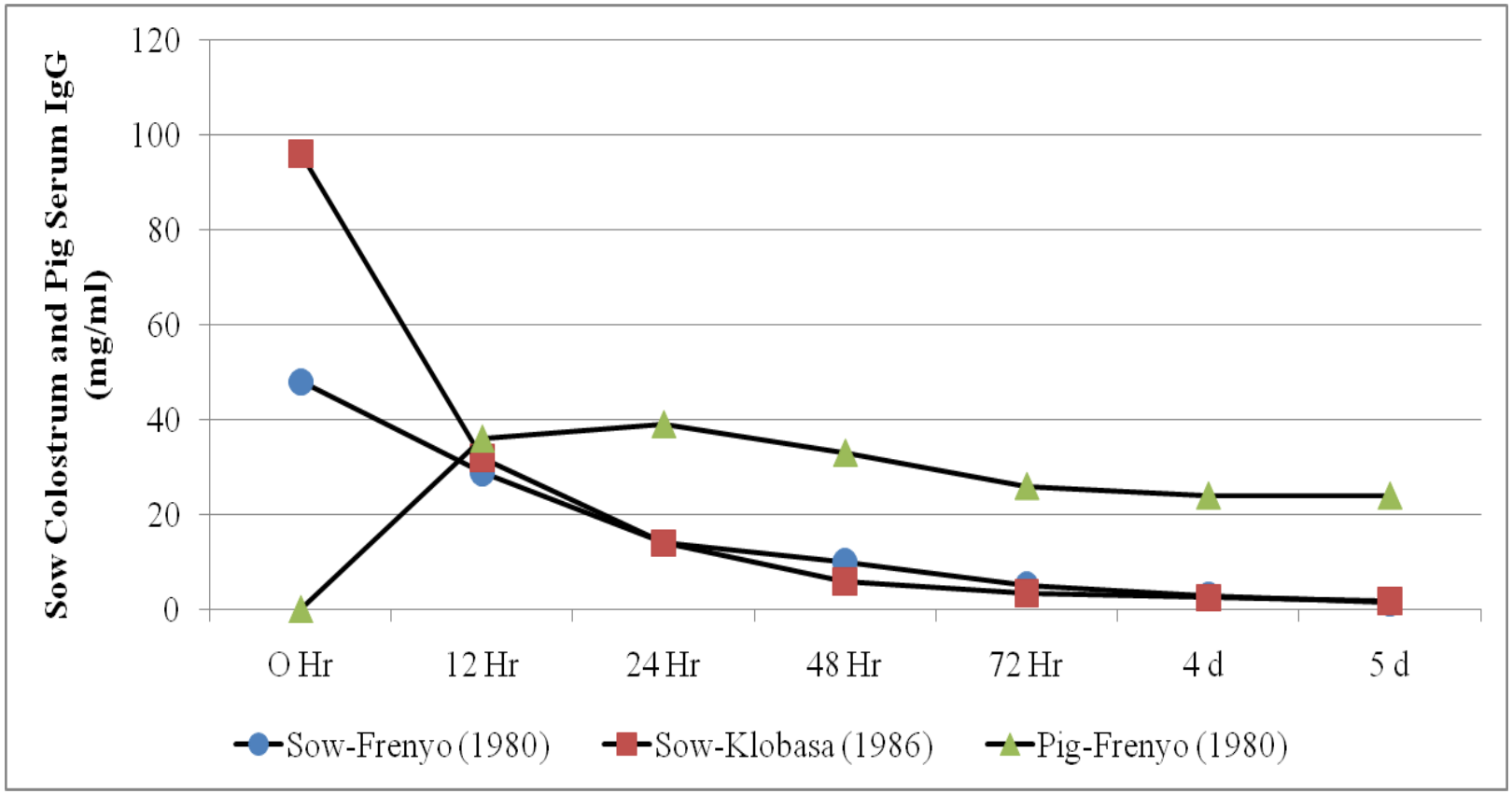

Figure 5. Changes in Sow Colostral IgG and Pig Serum IgG (mg/ml) Content in Swine as Function of Time.

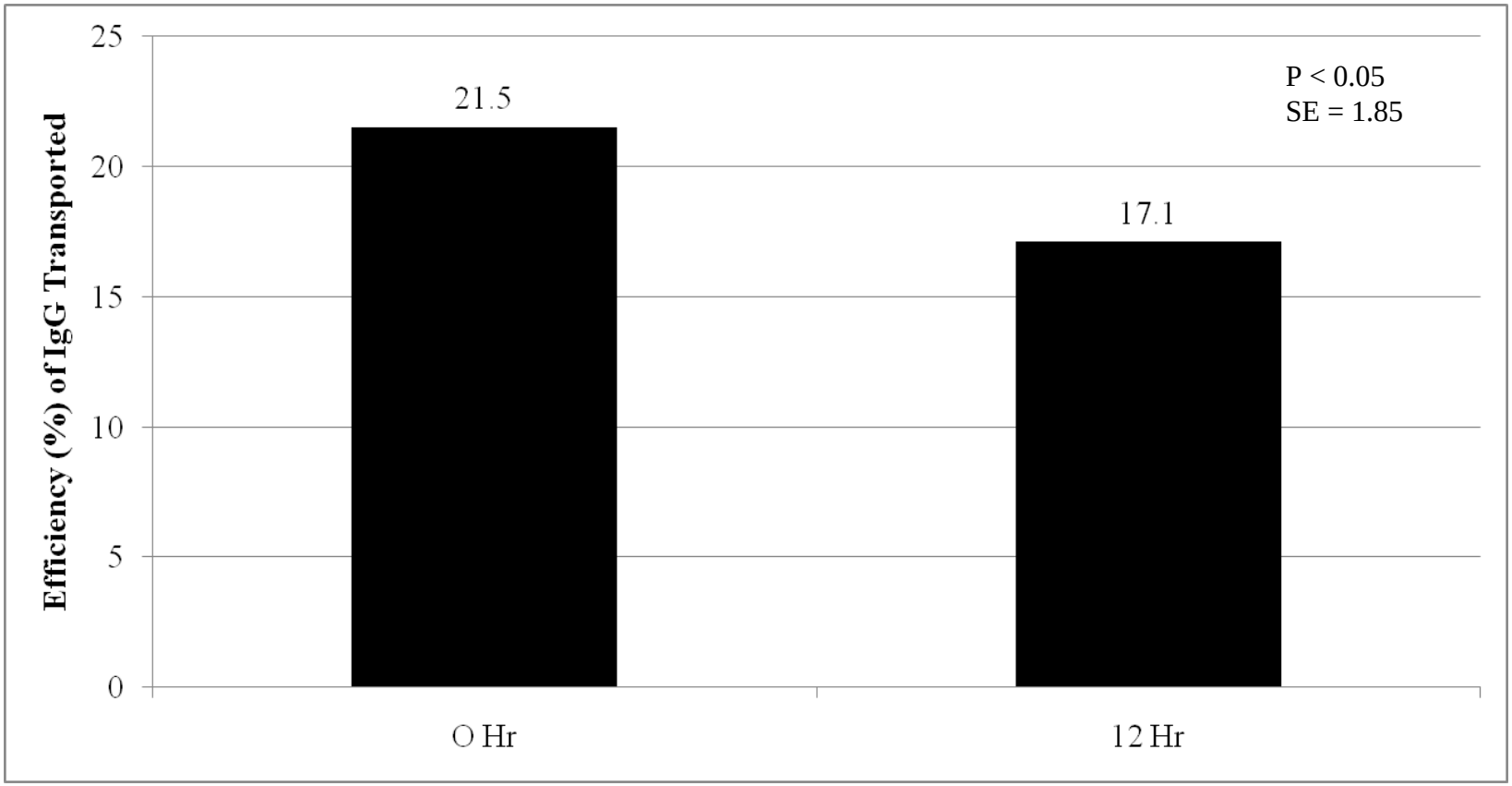

Figure 6. Efficiency (%) of IgG Transported by Piglets Gavaged with Colostrum at 0 h and 12 h of Age.

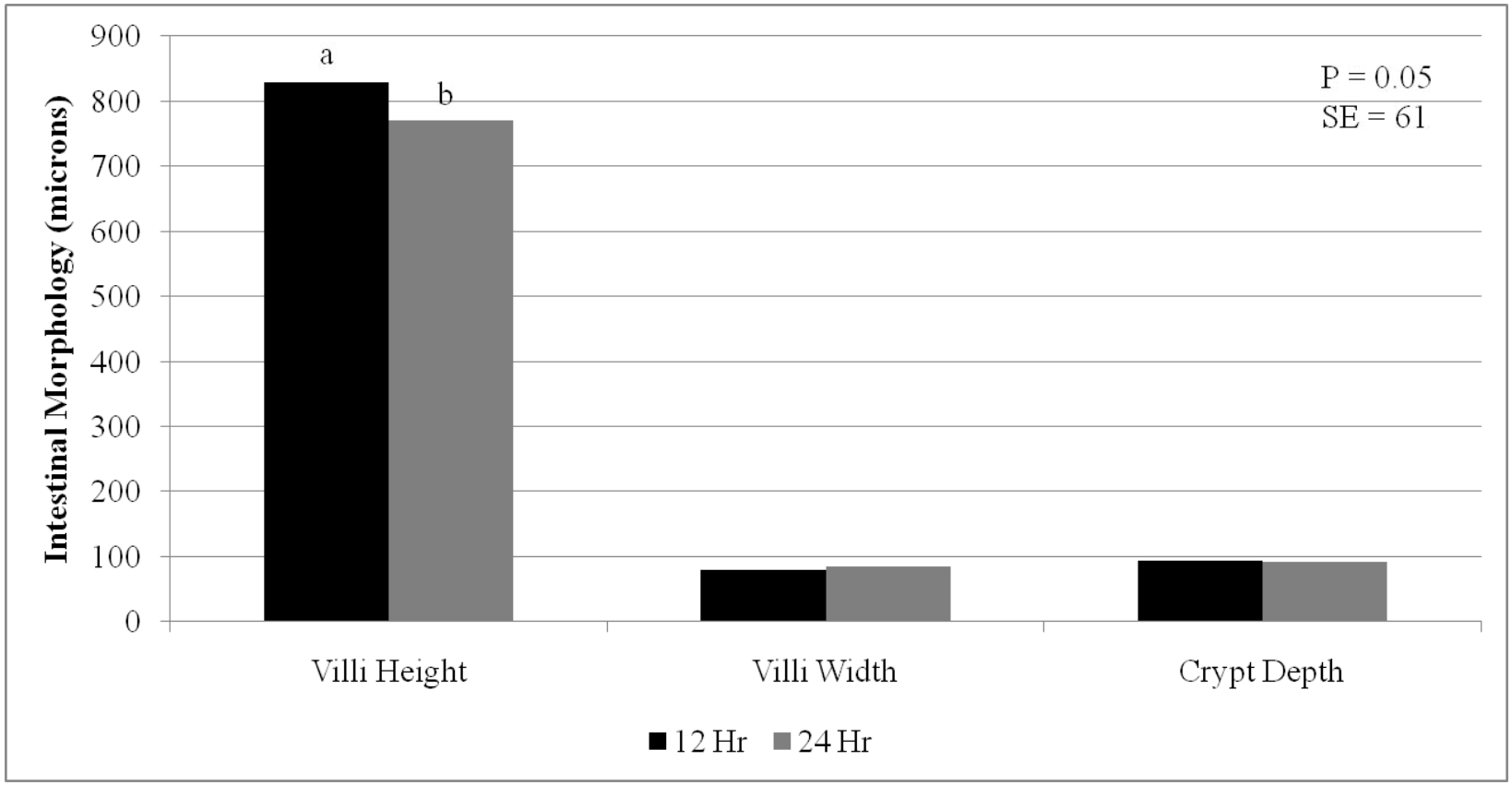

Figure 7. Intestinal Morphology (Microns) of 12-h and 24-h old piglet jejunum.

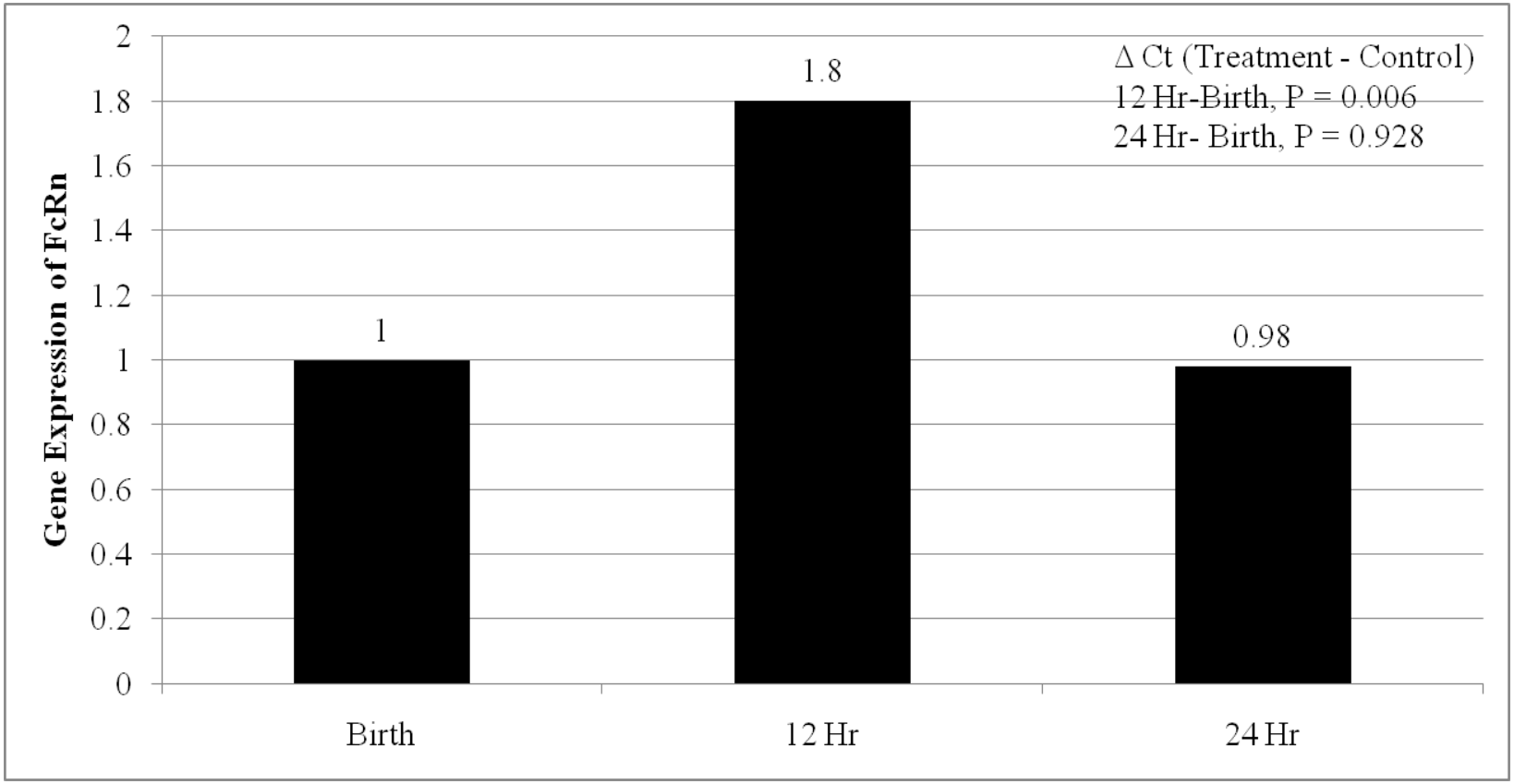

Figure 8. Relative gene expression of FcRn in pigs at 0 (birth), 12and 24 h of age.

Table 1: Real-time polymerase chain reactions (PCR) primers.

Genes	Primer Sequence	Accession #	Product Length (bp)
FcRn	[1]F: 5'-CCTCCTGATATACATGGC-3'	AY740682.1	82
	[2]R: 5'-TGAAACAATGAGAACACAAA-3'		
B_2M	F: 5'-GGCTGCTCTCACTGTCTG-3'	NM 213978.1	102
	R: 5'-AGTTCAGGTAATTTGGCTTTCC-3'		
GAPDH	F: 5'-ACACTCACTCTTCTACCTTTG-3'	DQ 845173.1	90
	R: 5'-CAAATTCATTGTCGTACCAG-3'		

[1]F = Forward, [2]R = Reverse.

LITERATURE CITED

1. Benevenga, N.J., J. Odle and G.L. Asche. 1992. Comparison of measured carbon dioxide production with that obtained by the isotope dilution technique in neonatal pigs: observations on site of infusion. J. Nutr. Volume 122, No. 11, pp. 2174-2182.
2. Blecha, F. 1998. Immunological aspects: comparison with other species. In: Verstegen, M.W.A., Moughan, P.J., Schama, J.W. (eds.), the lactating sow. Wageningen Pers, Wageningen, pp.23-44.
3. Bikker, P., G. Kranendonk, R. Gerritsen, L. Russell. J. Campbell, J. Crenshaw, C. Rodriguez, J. Rodenas and J. Polo. 2010. Absorption of orally supplied immunoglobulins in neonatal piglets. Livestock Science 134: 139-142.
4. Brambell, F.W. 1966. The transmission of immunity from mother to young and the catabolism of immunoglobulins. Lancet 2:1087-1093.
5. Brambell, F.W., W.A. Hemmings, and I.G. Morris. 1964. A theoretical model of gamma-globulin catabolism. Nature 203: 1352-1354.
6. Burmeister W.P., A.H. Huber and P.J. Bjorkman. 1994. Crystal structure of the complex of rat neonatal Fc receptor with Fc. Nature 372:379-283.
7. Butler, J.E., J. Sun, P. Weber, S.P. Ford, Z. Rehakova and J. Sinkora. 2002. Switch recombination in fetal porcine thymus is uncoupled from somatic mutation. Vet. Immunol. Immunopathol. 87:307-319.
8. Bustin, S.A. 2000. Absolute quantification of mRNA using real-time reverse transcription polymerase chain reactions. J. Mol. Endocrinol., 25, 169-193.

9. Carlile, A.E. and F. Beck. 1983. Maturation of the ileal epithelium in the young rat. J. Anat. 137:357-369.
10. Clarke, R.M. and R.N. Hardy. 1971. Histological changes in the small intestine of the young pig and their relation to macromolecular uptake. J. Anat. 108:63-77.
11. Chaudhury, C., S. Mehnaz, J.M. Robinson, W.L. Hayton, D.K. Pearl and D.C. Roopenian. 2003. The major histocompatability complex-related Fc receptor for IgG (FcRn) binds albumin and prolongs its lifespan. J. Exp. Med. 197:315-322.
12. Clarke, R.M and R.N. Hardy. 1971. Histological changes in the small intestine of the young pig and their relationship to macromolecular uptake. J. Anat. 108: 63-77.
13. Foss, D.L., M.J. Baarsch, and M.P. Murtaugh. 1998. Regulation of hypoxanthine phosphoribosyltransferase, glyceraldehydes-3-phosphate Dehydrogenase and beta-actin mRNA expression in porcine immune cells and tissues. Anim. Biotechnol. 9: 67-78.
14. Frenyo, V.L., G. Pethes, T. Antal and I. Szabo. 1980. Changes in colostral and serum IgG content in swine in relation to time. Vet. Res. Comm. 4: 275-282.
15. Garcia-Crespo, D., R.A. Juste and A. Hurtado. 2005. Selection of ovine housekeeping genes for normalization by real-time RT-PCR; analysis of PrP gene expression and genetic susceptibility to scrapie. BMC vet Res 1:3.
16. Ghetie, V. and E.S. Ward. 2000. Multiple roles for the major histocompatability complex class I-related receptor FcRn. Annu. Rev. Immunol. 18:739-766.

17. Hansen, S.L., M.S. Ashwell, L.R. Legleiter, R.S. Fry, K.E. Floyd and J.W. Spears. 2009. The addition of high manganese to a copper-deficient diet further depresses copper status and growth of cattle. Brit. J. Nutr.101:1068-1078.
18. Hardy. R.N. 1969. The absorption of polyvinylpyrrolidone by the newborn pig intestine. J. Physiol. 204: 633-651.
19. Jones, R.E. 1976. The selective uptake and transmission of proteins to the circulation from the small intestine of the suckling rat. Biochem. Biophys. Acta 451:151-160.
20. Jonsson, A. 1973. Transfer of immunoglobulins from mother to offspring in the pig. Acta vet. Scand. Suppl. 43:1-64.
21. Klobasa, F. J.E. Butler, E. Werhahn and F. Habe. 1986. Maternal-neonatal immunoregulation in swine: Influence of multiparity on the novo immunoglobulin synthesis by piglets. Vet. Immunol. Immunopathol. 11:149-159.
22. Kraehenbuhl, J.P. and M.A. Campiche. 1969. Early stages of intestinal absorption of specific antibodies in the newborn, J. cell. Biol. 42:345-365.
23. Leary, H.L. and J.G. Lecce. 1976. Uptake of the macromolecules by enterocytes on transposed and isolated piglet small intestine. J. Nutr. 106: 419-427.
24. Leary, H.L and J.G. Lecce. 1979. The preferential transport of immunoglobulins G by the small intestine of the neonatal piglet. J. Nutr. 109:458-466.
25. Lecce, J.C., G. Matrone and O.D. Morgan. 1961. Porcine neonatal nutrition: absorption of unaltered non-porcine proteins and polyvinylpyrrolidone from the gut of piglets and the subsequent effect of maturation of the serum profile. J. Nutr. 73: 158-166.

26. Lecce, J.C. 1973. Effect of dietary regimen on cessation of uptake by piglet intestinal epithelium (closure) and transport to the blood. J. Nutr. 103:751-756.

27. Le Dividich, R., J.A. Rooke, and P. Herpin. 2005. Nutritional and immunological importance of colostrum for the new-born pig. J. Ag. Sci. 143:1-17.

28. Livak, K.J and T.D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2 (-Delta Delta C_T method. Methods 25:402-408.

29. Lockey, C., E. Otto and Z. Long. 1998. Real-time fluorescence detection of a single DNA molecule. Biotechniques 24: 744-746.

30. Moon, H.W. 1971. Epithelial cell migration in the alimentary mucosa of the suckling pig. Proceedings of the Society of Experimental Biology and Medicine 137: 151-154.

31. Nygard, A-B, C.B. Jorgense, S. Cirera and M. Fredholm. 2007. Selection of reference genes for gene expression studies in pig tissues using SYBR green qPCR. BMC Molecular Biology 8:67.

32. Pfaffl, M.W., G.W. Horgan and L. Dempfle. 2002. Relative Expression Software Tool (REST©) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nuclei Acids Research. Volume 30, Issue 9, Page 36.

33. Rodewalt, R.B. 1973. Intestinal transport of antibodies in the newborn rat. J. Cell Biol. 58:182-211.

34. Rodewald, R. 1976. pH-dependent binding of immunoglobulins to intestinal cell of the neonatal rat. J. Cell Biol. 71:666-669.

35. Roopenian, D.C., G.J. Christianson, T.J Sproule, A.C. Brown, S. Akilesh, and N. Jung. 2003. The MHC class I-like IgG receptor controls perinatal IgG transport, IgG homeostasis and fate of IgG-Fc-coupled drugs. J. Immunol. 170:3528-3533.
36. Rundell, J.O. and J.G. Lecce. 1971. Mechanism of cessation of absorption of macromolecules (closure) in the neonate: Independence of closure and intestinal epithelial cell turnover. FASEB Federation Proceedings 30:236.
37. Schmittgen, T.D and K.J. Livak. 2008. Analyzing real-time PCR data by the comparative C_T method. Nature protocols. Volume 3. No. 6: 1101-1108.
38. Schnulle, P.M., and W.L. Hurley. 2003. Sequence and expression of the FcRn in the porcine mammary gland. Vet. Immunol. Immunopathol. 91: 227-231.
39. Simister, N.E. 2003. Placental transport of Immunoglobulin G. Vaccine 21: 3365-3369.
40. Simister, N.E. and K.E. Mostov. 1989. An Fc receptor structurally related to MHC class I antigens. Nature 337:184-187.
41. Smith, M.W. and L.G. Jarvis. 1978. Growth and cell replacement in the newborn pig intestine. Proceedings of the Royal Society of London Series B 203:69-89.
42. Smith, M.W. 1985. Expression of digestive and absorptive function in differentiating enterocytes. Annual Review of Physiology 47:247-259.
43. Staley, T.E., E.W. Jones and L.D. Corley. 1969. Fine structure of duodenal absorptive cells in the newborn piglet before and after feeding colostrum. Am. J. Vet. 30:567-581.

44. Steuerwald, N., J. Cohen, R.J. Herrera and C.A. Brenner. 1999. Analysis of gene expression in single oocytes and embryos by real-time rapid cycle fluorescence monitored RT-PCR. Mol. Hum. Reprod., 5, 1034-1039.

45. Stirling, C.M., B. Charleston, H. Takamatsu, S. Claypool, W. Lencer and R.S. Blumberg. 2005. Characterization of the porcine neonatal Fc receptor-potential use for trans-epithelial protein delivery. Immunology 114: 542-553.

46. Werhahn, E., F. Klobasa and J.E. Butler. 1981. Investigation of some factors which influence the absorption of IgG by the neonatal piglet. Veterinary Immunology and Immunopathology 2:35-51.

47. Wittwer, C.T., K.M. Ririe, R.V. Andrew, D.A. David, R.A. Gundry and U.J. Balis. 1997. The lightCycler: a microvolume multisample fluorimeter with rapid temperature control. Biotechniques 22: 176-181.

48. Ye, L., W. Tuo, X. Liu, N.E. Simister, and X. Zhu, 2008. Identification and characterization of an alternatively spliced variant of the MHC class I-related porcine neonatal Fc receptor for IgG. Developmental and Comparative Immunology 32: 966-979.

49. Zhu, X., J. Peng, R. Raychowdhury, A. Nakajima, W.I. Lencer and R.S. Blumberg. 2002. The heavy chain of the neonatal Fc receptor for IgG is sequestered in the endoplasmic reticulum by forming oligomers in the absence of β_2m association. Biochem J. 367:703-714.

50. Zhu, X., J. Peng, D. Chen, X. Liu, L. Ye and H. Lijima. 2005. Calnexin and ERp57 facilitate the assembly of the neonatal Fc receptor for IgG with β_2-microglobulin in the endoplasmic reticulum. J. Immunol. 175:967-76.

CHAPTER 5

EFFECTS OF CREEP FEEDING AND SUPPLEMENTAL GLUTAMINE OR GLUTAMINE PLUS GLUTAMATE (AMINOGUT) ON PRE- AND POST-WEANING GROWTH PERFORMANCE AND INTESTINAL HEALTH OF YOUNG PIGLETS

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ABSTRACT

Creep feeding is used as a management practice to stimulate post-weaning feed consumption. Glutamine is known to be a key source of fuel for intestinal epithelial cells, to regulate cell signaling via the mTOR pathway, and to stimulate cell proliferation, differentiation, migration, metabolism, homeostasis, survival and function. Due to these attributes, it has been associated with the reduction of villus atrophy during the first two weeks post-weaning. The objective of this study was to determine the impact of creep feeding and adding either L-glutamine (GLN) or AminoGut (AG) to pre- and post-weaning diets on pig performance and intestinal health. AG is the commercial name for the combination of glutamine and glutamic acid. Litters (N=120) were allotted to four treatments during 14-21 d of lactation: 1) Non-creep fed (NC, n=45); 2) creep fed control diet (CFCD, n=45); 3) creep fed 1% GLN (CFGLN, n=15); 4) creep fed .88% AG (CFAG, n=15). No effects of creep feeding on intake, weaning weight or mortality were detected (P>0.25). After weaning, the NC and CFCD groups were sub-divided into three groups (n=15 each), receiving either a control nursery diet (NC-CD, CFCD-CD) or a diet supplemented with either GLN (NC-GLN, CFCD-GLN) or with AG (NC-AG, CFCD-AG). The litters that were creep fed diets containing GLN or AG also were supplemented with those amino acids in the nursery diets (CFGLN-GLN, CFAG-AG). GLN was added at 1% in all three phases and AG was added at .88% in phase 1 and 2 and at .66 % in phase 3. Pigs were placed in a commercial nursery, housed 24 pigs / pen, and fed pelleted diets. Pigs receiving GLN in both pre- and post-weaning diets (CFGLN-GLN) had the best feed efficiency (gain/feed) among all the treatments for the first thee-week period (P<.056), exceeding controls (CFCD-CD) by 33%.

The NC-AG group had (P=0.02) the greatest feed intake among all treatments in the last three weeks of the study, exceeding controls (CFCD-CD) by 12%. CFGLN-GLN, CFCD-GLN and Sow Reared (SR) had the greatest ($P < 0.05$) villi height exceeding those which were creep fed with a control diet and later supplemented with AminoGut (CFCD-AG) by 18%, 20% and 19% respectively. SR group was added as a point of comparison with all the others treatments. We also found that pigs creep fed with a diet supplemented with AminoGut and also fed with a post-weaning diet supplemented with AminoGut (CFAG-AG) had the deepest ($P < 0.01$) crypts among all the treatments. CFGLN-GLN, CFCD-GLN and SR groups had the greatest ($P < 0.0001$) number of cells proliferating (PCNA) height exceeding those which did not receive creep feed and later receiving a control diet (NC-CD) by 43%, 54% and 63% respectively. Sow reared (SR) pigs showed the greatest ($P < 0.0001$) intestinal absorption capacity for xylose and mannitol. There was no significance difference among the others treatments on the absorption of these sugars. We found no significant differences among all treatments in maltase activity. In conclusion, supplementation of creep feed and nursery diets with GLN and/or AminoGut in the first three weeks improved feed conversion possibly due to improved intestinal health.

INTRODUCTION

After pigs are weaned from their dams, morphological and functional changes take place in their small intestine. Pluske et al, (1997) has reported decreased villi height and increased crypt dept. Li and others (1991) reported that inclusion of soybean meal in starter pig diets may be responsible for shortened intestinal villi height and hypertrophy in the crypt.

Enzymatic profile changes have been reported with a decrease in the activity of the brush-border enzyme lactase and increase activities of maltase and sucrase (Autamire and Corring, 1978; Cera et al., 1988a). Pancreatic α-amylase and maltase II and III production increase with age (Longland, 1991) but may not be adequate at 4 to 5 wk of age to adapt to the more complex ingredients from a corn-SBM diet (Mahan and Newton, 1993). Because pigs are being transitioned from milk to dry feed, the pig's GIT tract is unable to fully digest the macronutrients from the feed or absorb all of what has been digested.

Various researchers (Kentworthy and Crabb, 1963; Palmer and Hulland, 1965) have reported that this accumulation of undigested and unabsorbed feed creates the perfect medium for opportunistic bacteria such as hemolytic *E. coli* to grow. The normal weaning process will stimulate pancreatic development and its enzymatic output; however there is a delay until the different enzymes reach efficient levels (Cera et al., 1990). This in turn can cause post-weaning diarrhea. We hypothesized that creep feeding in suckling piglets and adding glutamine to pre and/or post-weaning diets would alleviate villi atrophy and support normal growth.

Creep feeding is deemed to be very important during the suckling period for swine practitioners because it (1) increases weaning weight when offered in small and frequent quantities and (2) eases the transition period for the piglets from sow's milk to the dry feed. The latter has a physiological implication in order to avoid digestive upset such as post-weaning diarrhea and poor growth.

Some argue (Newby et al., 1983; Miller et al., 1984) that the use of creep feed during the suckling period could potentially trigger hypersensitivity to feed antigens and that can

stimulate post-weaning diarrhea. Barnett et al. (1989) observed antibody titers in the blood of weaned piglets and confirmed that feed antigens can induce an immune reaction in creep-fed pigs.

The creep feed is typically a complex diet containing high lactose and highly digestible ingredients (rolled steamed oats versus corn). Due to this complexity, this diet is not only very expensive but also slows down the normal feed production schedule due to the small amounts being produced-- therefore the reluctance of most swine practitioners to produce it. The reduction in feed intake associated with weaning has been known to affect intestinal integrity and potentially cause pathological disorders. Klasing (2007) argued that dietary supplementation of some nutrients or immune modulators can rectify the intestinal impairment and modulate the immune function of animals contributing to the overall health and performance.

Nutrition can regulate the type of immune response by a number of mechanisms (Klasing, 2007). Swine nutritionists have traditionally focused on those amino acids (AA) that can't be synthesized by the animals without paying too much attention to those that can be synthesized by the animals and have a tremendous impact on regulating nutrient metabolism and the immune responses (Li et al., 2007, Wu, 2009). These AA include arginine, glutamine, glutamate, proline, leucine, cysteine and tryptophan. Recent studies indicate that these AA serve important regulatory functions in nutrient metabolism, protein turnover, and immune function, thereby enhancing growth and feed efficiency in pigs. The underlying mechanisms include activation of nitric oxide, mammalian target of rapamycin

(mTOR), gaseous signaling, and AMP-activated protein kinase pathways as well as anti-oxidative (Wu, 2010).

The concentration of glutamine (Gln) in the body ranges from 2 to 15 mM (Meijer et al., 1993; Le Boucher et al., 1997) making it the most abundant AA. It plays a central role in interogan N transfer and regulation of intermediary metabolism (Haussinger et al., 1994). Gln is a major metabolic fuel for rapidly dividing cells, including enterocytes and lymphocytes, as well as a key regulator of gene expression and cell signaling pathways (Rhoads and Wu, 2009). Schock and Goldstein (1981) reported that glutamine serves as precursor for the increased renal ammoniagenesis during chronic metabolic acidosis. The amide nitrogen of glutamine is essential for purine and pyrimidine biosynthesis.

Gln has important and unique metabolic functions, and it is considered a conditionally essential amino acid in some species under inflammatory conditions (Newsholme, 2001) and disease states (Napper et al., 1997; van de Poll et al., 2004). Souba and others (1990) have indicated that the provision of Gln-enriched diets in various stress states associated with bacterial translocation decreases the incidence of translocation of bacteria by decreasing the adherence of bacteria to enterocytes.

Reeds and others (2000) argued that the high metabolic rate of the intestinal mucosa is very unique when compared to the others organs in the body. First, the enterocytes are specialized on absorbing nutrients from the lumen to the basal lamina. Second, mucosa cells are presented with high quantities of substrates from both the intestinal lumen and the mesenteric arterial circulation. Under fed conditions, the quantification of substrate used by

the gut can be complicated to quantify given the fact that potential oxidative precursors are supplied from both the diet and the arterial circulation.

Finally, Gln is the only AA in arterial blood that is taken up by the small intestine in swine (Wu et al., 1994a). The small intestine (even though only represents 3 to 4% of the body weight) utilizes 30% of the arterial Gln and 67% of dietary Gln in swine. For comparison, 95 to 97% of dietary glutamate is extracted by the pig small intestine in first pass (Stoll and Burrin, 2006; Wu et al., 2010a) but only 50% is metabolized to CO_2 (Reeds et al., 2000).

Because the gastrointestinal tract has an obligatory requirement for glutamine (Reeds et al.,2000) and availability of glutamine from endogenous tissue production may not be sufficient for the maintenance of the structural and functional integrity of the intestinal mucosa (van der Hulst et. al., 1993; James et al., 1998), we hypothesized that the addition of L-glutamine or the combination of L-glutamine and glutamate may avoid gut atrophy which will result in the dysfunction of its digestive, absorptive and barrier functions. The objective of the study was to evaluating the effects of L-glutamine and AminoGut (L-glutamine and L-glutamate) on intestinal histology, intestinal absorptive capacity, enzymatic activity, and growth performance in a commercial swine operation. The effects of both products on piglet growth performance have not been evaluated during the whole nursery period when both have been offered during the pre-weaning period.

MATERIALS AND METHODS

The study was conducted during the summer 2010 (June 14th though August 27th) on a 4800-sow farm in Maple Hill, NC (Murphy-Brown, LLC; Rose Hill, NC). One day post birth, the whole litter of pigs was weighed using a "WayPig" litter scale (model 252, serial 26116) from Raytec Manufacturing (Ephata, Pennsylvania). Pigs were weaned at an average of 20-21 d of age and creep fed from d 14 until weaning. The pelleted creep feed used was a phase 1 diet and manufactured at the NCSU feed mill. Litters were creep fed every 4 h (8:00 AM, 12:00 and 4:00 PM) and were properly identified with colored ribbons for the four different treatments (No Creep Feed, Creep Feed, Creep + L-glutamine and Creep + AminoGut).

At weaning pigs were transported to site 2 (~ 300 meters from Site 1) and four days post-weaning, the whole pen of pigs was weighed using a scale (model 640) from Avery Weight-Tronix (www.agscales.com, Fairmont, MN). In Site 2, all treatments were also properly identified with colored ribbons and hand-fed for the next three weeks. Pigs were fed on an as-need-basis and had feed in the feeder for 24 h. After three weeks, the pens were weighed for growth and feed conversion calculation. After that, two adjacent pens shared a feeder until weaning.

After one week post weaning, one pig per treatment was fasted overnight, gavaged with a D-xylose/mannitol solution and consequently euthanized for tissue collection the next day. A solution containing 0.2 g/mL of D-xylose (Pfizer, N.Y., NY) and 0.3 g/ml of mannitol (Sigma, Saint Louis, MO) was prepared and was given to the pigs on an average of 9 h and 20 minutes after fasting. The selected dose was 6.5 mL/kg of body weight. Pigs were

individually weighed using a Berkley FS-50 hanging scale from Tackle-Direct (Somers Point, NJ). Two h post gavage; pigs were bled via jugular venipunture using a vaccutainer tube with a 20 x 1.5” needle. The time of bleeding and post-gavaged of the xylose/mannitol solution was adapted from Doerfler et al. (2000). After pigs were bled they were humanly euthanized for tissue collection. Jejunum samples (25 cm from the stomach) were collected for H & E staining and for Scanning Electron Microscopy (SEM). The portion cut specifically for SEM, was cut open and laid flat in a small cartridge in order to obtain a better picture of the intestinal villi. A separate jejunum tissue sample was cut and the intestinal mucosa was scrapped for maltase activity analysis. Blood was centrifuged the next day and the serum stored at –20 °C for further analysis. Performance data were statistically analyzed using the PROC GLIMMIX of SAS with birth weight and wean age as covariates. Histology data were analyzed using the Mixed Procedure of SAS with the body weight (one week post-weaning in the nursery) as covariate.

Scanning Electron Microscopy: Scanning Electron Microscopy (SEM) is a technique that allows you to observe the surface of the gut epithelium in three dimensions. Jejunum samples were collected from one-week old piglets and immersed in McDowell's and Trump's 4F:1G fixative containing 4% formaldehyde and 1% glutaraldehyde in a phosphate buffer, with an osmolarity of 176 mOsM and a pH of 7.2-7.4 (McDowell and Trump, 1976). Samples were cut to be between 2-3 mm in order to minimize chances of bulk charging. Samples were rinsed in 0.1 M phosphate buffer and dehydrated in an ethanolic series to 100% ethanol before subjection to critical point drying after being stored for approximately 7 wks in the 4F: 1G fixative. Samples were then mounted on SEM stubs with carbon tape and

sputter coated with gold-palladium before being viewed with a JEOL JSM-6360LV scanning electron microscope (JEOL, Peabody, Massachusetts). This microscope is a fully digital instrument that can view specimens by secondary electron imaging (SEI), backscatter electron imaging (BEI), at high vacuum, or at low vacuum.

Hematoxylin & Eosin Staining: This procedure was carried-out as described in Chapter 4.

PCNA Staining: Proliferation cells nuclei antigen (PCNA) is a procedure that stains proliferating cells. Five micron jejunal slices were mounted on glass slides. A primary mouse monoclonal antibody (PC10) was used as a proliferation marker. This antibody is specific for PCNA p36 protein expressed at high levels in proliferating cells. It was diluted at 1:1500 and incubated for 30 minutes. The remaining steps were completed using the Dako EnVision Mouse kit (Dako, Denmark). Intensively stained and the total number of enterocytes were counted in 8 consecutive well-orientated crypts (those that extended to the muscularis mucosa).

Analysis of Mannitol*:* Samples of serum were frozen, thawed at room temperature and vortexed to mix. Samples were then filtered by centrifugation using Ultrafiltration Spin Columns (045 micron, Millipore, Temecula, CA). An aliquot of 200 µL of sample was transferred to HPLC autosampler vials containing 250 µL inserts. An internal standard solution of myo-inositol was added (2 µL). Analysis was done using High Performance Liquid Chromatography (HPLC).

The extracts were analyzed using a Dionex BioLC (Dionex Corporation, Sunnyvale, CA) at a controlled temperature of 30°C. The system consisted of a gradient pump, an autosampler,

and a Pulsed Amperometric Detector (PAD). The mobile phase was 52 mM NaOH (Thermo-Fisher Chemical Corp. Pittsburgh, PA) at an isocratic flow rate of 1.0 mL/min. The column used was a Dionex PA-10, 250 mm length and 4 mm i.d., fitted with a Dionex PA-10 Guard column and a Dionex Borate Guard column. The detector was programmed to run a quadruple waveform as recommended by the manufacturer. A shift in the detector range was 1 micro Coulomb. The injection volume was 10 µL. The mannitol was calculated using an authentic standard of d-mannitol and myo-inositol as an internal standard. All the reference standards were purchased from Sigma Chemical Corp (St. Louis, MO).

Analysis of Xylose: The collected pig serum (20 µL) was subjected to a modified micro method (Goodwin et al., 1984a, b) first described by Eberts et al. (1979) for determination of plasma D-xylose. To each 20 µL serum sample, 2 mL of phloroglucinol (Sigma Chemical Co., Saint Louis, MO 63178-9916) color reagent was added and heated for 4 min at 100º C. The samples were allowed to cool at room temperature in a water bath. After cooling, the absorbance of each sample was read on a Gilford UV-Vis spectrophotometer (Thermo Fisher Scientific, Inc.; Waltham, MA) set at 554 nm.

Maltase Enzyme Activity: Maltase in the presence of maltose will liberate 2 units of glucose. Maltase activity is produced from the proteins sucrase-isomaltase (90% of maltase activity) and maltase-glucoamylase (10% of maltase activity). This assay does not distinguish between the two separate proteins. The principle of this reaction is as follows:

Maltose + (n-1) H_2O --[Maltase in sample]--→ n glucose

B-D-glucose + O_2 + H_2O --[Glucose Oxidase]--→ D-gluconic + H_2O_2

H_2O_2 + o-dianiside (reduced colorless) --[Peroxidase]--→ o-dianiside + H_2O (oxidized red color).

The maltase assay was performed as described by Dahlqvist (1984). Maltase activity (U/g of protein) is expressed as units, with 1 unit defined as the amount of enzyme transforming 1.0 µmol of substrate per min at 25 ºC.

Statistical Analysis: All variables were analyzed using the PROC MIXED procedure of SAS. Litters were used as the experimental unit during the pre-weaning period and pens were used as the experimental unit during the post-weaning period. The LS means were calculated for all treatments and $P < 0.05$ was used to determine significant differences among the treatments.

RESULTS

Results for pre-weaning performance are summarized in Table 1. We found weaning age to be significant ($P < 0.0001$) among the pre-wean treatments. For subsequent comparisons, this variable was used as covariate. Pigs/litter, sow parity, birth weight, weaning weight, and mortality were not different among the treatments. Creep feed consumption also did not differ for those treatments receiving creep feed. The average creep

feed consumptions for the control diet and those supplemented with either glutamine or AminoGut were 49.44, 45.57 and 48.44 g/pig respectively. We did not find an effect of creep feeding on post-weaning performance. A longer (> 1 wk) creep feed period needs to be examined.

Pigs receiving GLN in both pre- and post-weaning diets (CFGLN-GLN) had the best feed conversion (feed/gain) among all the treatments for the first thee-week period (P<.056), exceeding controls (CFCD-CD) by 33% . All others variables were not significant during this post-weaning period among the treatments.

The NC-AG group had (P=0.02) the greatest feed intake among all treatments in the last three weeks of the study, exceeding controls (CFCD-CD) by 12%. All others variables were not significant during this post-weaning period among the treatments.

CFCD-GLN, Sow-Reared and CFGLN-GLN groups had the greatest (P < 0.05) villi height exceeding those which were creep fed with a control diet and later supplemented with AminoGut (CFCD-AG) by 20%, 19% and 18% respectively. The Sow-Reared group was added as a point of reference against the other treatments. All tissue samples for all treatments were taken at 28 d of age.

We also found that pigs creep fed with a diet supplemented with AminoGut and fed a post-weaning diet supplemented with AminoGut (CFAG-AG) had the deepest (P < 0.0001) crypts among all the treatments.

Sow-Reared, CFCD-GLN and CFGLN-GLN, and groups had the greatest (P < 0.0001) number of cells proliferating (PCNA), exceeding those which did not receive creep feed and later receiving a control diet (NC-CD) by 63%, 54% and 43% respectively. We

found a correlation between villi height and PNCA: the taller the villi height, the greater the number of proliferating cells.

Sow-Reared pigs showed the greatest ($P < 0.0001$) intestinal absorption capacity for xylose and mannitol when compared with the others treatments. The levels of xylose and mannitol found in the sow reared pigs blood exceeded the average of the levels found in the other treatments by 3.2 and 7.4 fold respectively. This is consistent with the architecture of the villi of the sow reared pigs when compared to the other treatments. There was no significance difference among the other treatments on the absorption of these sugars. We found the levels of xylose in the blood to be higher than those of mannitol even though a higher concentration of mannitol was contained in the final solution (0.2 g/L vs. 0.3 g/L). We found no significant differences among the treatments in maltase activity although there was a tendency ($P = 0.18$) for creep fed treatments to be numerically different than those which did not receive creep feed (260 vs. 214 µmoles / (min. g of protein respectively)).

DISCUSSION

Windmueller and Spaeth determined that in the adult rat small intestine, CO_2, lactate, alanine and glucose account for 56-64, 16-20, 4-8, and 2-10% of the total catabolized carbons of luminal glutamine, glutamate and aspartate, respectively. These results and others showed that amino acids (glutamine, glutamate and aspartate), rather than glucose, are the major fuels for the small intestine mucosa, responsible for providing energy required for intestinal ATP-dependent metabolic processes (Burrin and Reeds, 1997). Although there seems little doubt that glutamine plays an important, but remarkably poorly characterized

role in the metabolism of many proliferating cells, much of the more recent literature on intestinal metabolism has ignored two observations made by Windmueller and Spath (1975). Those are, first, that the metabolism of luminal glutamate was even more extensive than that of arterial glutamine; and second, that the presence of high concentrations of glutamate in the intestinal lumen had only a small (less than 25%) effect on intestinal utilization of glutamine. This perhaps suggests that these two closely related amino acids may have different functional roles in the intestinal mucosa.

Reeds and others approached the question of quantifying those compounds used to generate ATP in the gut. They combined measurements of portal nutrient balance with enteral and intravenous infusion of [U-^{13}C] substrates (Glutamate, Glucose and Glutamine) in rapidly growing pigs that were consuming diets based on whole-milk proteins. The results revealed 95% of the dietary glutamate presented to the mucosa was metabolized in first pass and that of this, 50% was metabolized to CO_2. Dietary glucose was oxidized to a very limited extend, and arterial glutamine supplied no more than 15% of the CO_2 production by the portal-drained viscera. Glutamate was the single largest contributor to intestinal energy generation. The results also suggested that dietary glutamate appeared to be a specific precursor for the biosynthesis of glutathione (protection of the mucosa), arginine and proline by the small intestinal mucosa. Furthermore these functions are apparently different from those of arterial glutamine, the substrate that has received the most attention.

It seems that glutamate can partially substitute for Gln in several pathways, including ATP production and synthesis of arginine, alanine, proline and aspartate (Reeds et al., 1997; Wu, 1998). Glutamate plays a significant role on avoiding Gln degradation by mitochondrial

phosphate-activated glutaminase in extra hepatic tissues and cells (Curthoys and Watford, 1995) yielding a sparing effect on the use of Gln as a metabolic fuel (Yin et al., 2010) and its availability in cells (Boutry et al., 2011). Wu (2009) argued that that key functions of Gln (syntheses of Gln-tRNA, aminosugars, carbamoylphosphate, NAD, NADP, as well as purine and pyrimidine; renal ammoniagenesis; and regulation of ODC expression) cannot be made by glutamate. He and others (1994a) argued that although both Gln and glutamate provided in the enteral diet are extensively catabolized by the small intestine, this organ takes up Gln, but not glutamate, from the arterial blood. In a separate study, Wu and others (1994b) argued that due to the complex compartmentalization of cellular metabolism, extracellular glutamate may channel preferentially into the cytoplasm rather than into the mitochondria and, therefore, have different effects than the glutamate generated from Gln in mitochondria.

The required percent of supplemental Gln in the diet was adapted by Wu and others (2010a). They have calculated that the Gln requirement of the small intestine (965 mg/kg BW/d) in the 21-to 35-d-old sow reared piglet can be used as an ideal reference for that in the age-matched post-weaning pigs. However, the provision of Gln in the diet plus arterial blood is only 618 mg/kg BW/d. Thus, the need for supplemental Gln in the diet is 347 mg/kg BW/d. They then concluded that based on feed intake (33 g/kg BW/d) of 21-to 35-d-old post-weaning pigs, the required percent of supplemental Gln in the diet is 1.05% (0.347/33 x 100 = 1.05%).

Recent studies have shown that specific AA such as glutamine and arginine can promote gastrointestinal integrity across species (Ziegler et al., 2003; Clifford, 2006). Chamorro et al., (2010) have shown that 1% supplementation of L-glutamine to post-weaned

(25 d to 56 d of age) rabbit diets decreased fattening mortality and modified the intestinal microbiota but no consistent effects were observed on mucosal histology or on inflammatory and systemic immune responses.

The vast majority of research showing the benefits of supplementing Gln in the diet can be found in swine. Wu et al. (1999) reported that among all the amino acids, uterine and umbilical uptake of Gln was the greatest in pregnant gilts, implicating an important role for Gln in fetal growth and development. They fed 1% Gln in the diet of gestating gilts between 90 and 114 d of gestation and found that it significantly increased average birth weight. They also found that the number of intrauterine growth retarded piglets, variation in birth weight and pre-weaning mortality were reduced by 39, 33, and 46%, respectively, when compared with the control group.

Kim and Wu (2009) reported that lactating sows have a high requirement for Gln. Li et al. (2009b) reported that the uptake of Gln by the porcine mammary glands may be inadequate for the synthesis of milk proteins. By d 10 during the lactating period, the mammary glands uptake 16 g Gln/d from the arterial circulation (Trottier et al., 1997), however Haynes and others (2009) reported that at that point in time, 36 g Gln/d is being secreted. Wu and colleagues (1999) fed 1% Gln from d1 to d 20 to lactating sows and found an increase of Gln concentrations in the plasma, skeletal muscle and whole milk of the sows, as well as piglet growth and survival.

Haynes et al., (2009) evaluated the effectiveness of Gln or L-alanyl-L-glutamine (Ala-Gln) in vivo with 7-d-old piglets challenged with a single intraperitoneal injection LPS (0.1 mg/kg body weight). Administration of Gln or Ala-Gln to LPS challenged piglets

increased Gln concentrations in small intestinal lumen and plasma, reduced intestinal expression of TLR-4, active caspase-3 and NF*k*B, ameliorated intestinal injury, decreased rectal temperature and enhanced growth performance. These results demonstrate a protective effect of Gln or Ala-Gln against LPS-induced enterocytes death. They also reported that the Gln supplementation stimulated the growth of sow-reared piglets by 12%.

Yi et al. (2005) found that feeding glutamine had beneficial effects in alleviating growth depression of *E. coli* $K88^{+}$-challenged pigs, mainly via maintaining intestinal morphology and function, and/or possible modulating the somatotrophic axis. Jiang and others (2009) reported similar results.

Wu et al. (2010b) orally administered Gln (0.5g/kg BW/d) to low-birth weight piglets from 0 to 21 d of age and found that their growth were improved by 16% and their pre-wean mortality by 48%.

Our results are most consistent with those reported by Wu et al., (1996). They found a 29% improvement in feed conversion (21d post-weaning) when supplementing 1% glutamine. Glutamine (Gln) supplementation (1%) prevented jejunal atrophy (measured as villus height) during the first week post-weaning and increased feed: gain ratio (indicator of growth performance) by 25% during the second week post-weaning. It also increased plasma concentration of aspartate, glutamate and alanine and also reduced the extent to which plasma taurine concentration fell in post-weaning pigs. The prevention of villi atrophy during the first week post-weaning also has been reported by Wang and co-workers (2008a).

Liu and others (2001) reported similar results than those reported by Wu et al. (1996). They fed 1% L-glutamine or 1% L-glutamate to weaned pigs from 28-d to 42-d of age.

Jejunal atrophy was prevented during the first week for the groups fed either L-glutamine or L-glutamate when compared to the control group. Again these results provide an experimental basis for the use of glutamine and glutamate to improve piglet intestinal health and to support improved growth performance.

Xylose (greek *xylos*, "wood") is a sugar first isolated from wood, and named for it. Xylose is classified as a monosaccharide of the aldopentose type, which means that it contains five carbon atoms and includes an aldehyde functional group. It is the precursor to hemicellulose, one of the main constituents of biomass. Like most sugars, it can adopt several structures depending on conditions. With its free carbonyl group, it is a reducing sugar. D-Xylose absorption test has been used as a tool for the assessment of the effect of anticoccidials on the intestinal absorptive capacity of broilers during experimental coccidiosis (Mansoori et al., 2008) and malabsorption in poult enteritis and mortality syndrome (Doerfler et al., 2000). D-xylose, a poorly metabolized pentose sugar, is well absorbed from the small intestine of chickens and readily excreted in the urine (Doerfler et al., 2000). Blood D-xylose concentrations are expected to peak at 30-60 min after intake in poultry (Doerfler et al., 2000; Mansoori et al., 2008) and 60 min in pigs (You-Sheng et al., 1998; Chang et al., 2006).

Mannitol ($C_6H_{14}O_6$) is a highly hydrophilic, open-chain polyalcohol, that has been found in bacteria (formed mainly by *Leuconostoc mesenteroides*), algae, fungi and higher plants where it functions as a substrate of the carbon and energy metabolism as well as osmoregulator and antioxidant (Jennings et al., 1998; Shen et al., 1999). It tastes about half as

sweet as sucrose (Poulton and Winterborn, 1987). It is derived from the hydrogenation of glucose or fructose and is frequently used as an additive in the food as well as the pharmaceutical industry (Le and Mulderrig, 2001; Burger et al., 2000). Mannitol has been clinically applied in diagnostic and therapeutic doses for 1) the determination of extracellular fluid volume and glomerular filtration rate, 2) testing intestinal absorption and mucosal integrity, 3) as a diuretic and 4) as a radical scavenger and osmotically active component of infusions.

The pentose sugar xylose is absorbed across the gut wall of hamster and rats (Alvarado, 1966; Salem et al., 1965). However, xylose is not rapidly metabolized in mammalian tissues, and in humans this is the basis for the xylose absorption test for intestinal disease (Zilva and Pannall, 1984). Ruminants are the only mammals that have been shown to use xylose efficiently. The metabolism of xylose by ruminal bacteria is quicker and more efficient than that of the vertebrates. Xylans are xylose polymers that are major components of the hemicellulose found in plant cell walls (Johnson et al., 1999). When the ruminants eat plant material, the bacteria in the rumen degrade the xylan to xylose and then metabolize the xylose.

The relative small, hydrophilic marker molecule ^{14}C-mannitol has been shown to pass the epithelium by passive diffusion influenced by solvent drag, thus following the net flow of water (Menzies, 1984). The reduced permeability of ^{14}C-mannitol observed in the proximal jejunum after lectin treatment may be explained by a reduction in the absorptive surface area caused by villi shortening.

There are few studies using these two sugars as markers of gastrointestinal in vivo permeability in pigs in a commercial setting. In this study, the uptake of xylose was greater than mannitol regardless of their molecular weight (150 and 182 g/mol respectively) and the amount administered (mannitol higher than xylose). As discussed earlier, xylose can be metabolized in the gut by bacteria, and then absorbed whereas mannitol cannot. Therefore we would expect xylose to be absorbed more rapidly than mannitol. Mannitol is partially metabolized, the remainder being excreted in the urine. Nasrallah and Iber (1969) administered orally doses of 20 to 100 g of ^{14}C-mannitol to five humans with cirrhosis of the liver and to five subjects who had normal liver function. They found that at least one-sixth of orally ingested mannitol is absorbed and about one-third is metabolized. Laker and others (1982) found that mannitol occurs naturally in human urine.

The lack of significant differences in performance among the treatments for the entire 6-wk period correlates well with the lack of significant differences among the treatments for the levels xylose and mannitol absorbed and found in the their blood. We were not surprised by the high levels of intestinal absorptive capacity shown by the sow reared pigs when compared to the other treatments. These pigs have not experienced the different stress factors associated with weaning.

In young animals, lactase activity prevails, however as it gets older then maltase activity (as well amylases, lipases proteases) increases. Low concentration of maltase in the surface of epithelial cells may be an indication of villus atrophy due to disease or malnutrition (Kidder and Manners, 1978).

Kidder and Manners (1980) investigated the levels of the brush-border enzymes isomaltase (oligo-1,6-glucosidase), maltases 2 and 3 (glucoamylase) in the small intestines of eighty-four pigs of different ages ranging from 3 weeks to 4.5 years. The levels of isomaltase increased with age throughout the age-range studied. Glucoamylases increased with age up to 200--300 d of age. The pattern of distribution of the brush-border enzymes altered with age in the piglets, but approached the adult pattern by 8 weeks. Piglets weaned at 3 weeks had higher levels of isomaltase and glucoamylases at 5 weeks than piglets left on the sow. At 8 weeks of age the piglets weaned at 3 weeks still had higher isomaltase levels than those on the sow. In our study, pigs weaned at 3 weeks of age and later placed in the nursery for 7 d, did not have higher maltase activity than those that suckled the sow for 28 d.

Thomsson et al., (2007) reported maltase activity tended to be greater in the cranial section of the small intestine for pigs fed with phytohemagglutinin (PHA) which is a lectin obtained from red kidney beans (*Phaseolus vulgaris*). Similar results with PHA fed-pigs were reported by Radberg et al., 2001. They reported increased maltase activity (U/g of protein) in the middle and distal jejunum. Bikker et al., (2006) reported that maltase activity tended to decrease with increasing dietary fermentable carbohydrates. We were unable to find any significant differences among the treatments in maltase activity.

Scanning electron microscopy (SEM) allows observation of the surface of the epithelium in three dimensions and gives a fresh dimension in the investigation of gut mucosa (Skrzypek et al., 2005). The visual assessment of the SEM graphs showed that pigs which were not creep fed during the suckling period had a rough villi surface with numerous

cells shedding (apoptosis) along the entire length of the villi (figure 1). They also showed deep transversal furrows in most (if not all) the epithelial cells (figure 2 and 3). Those treatments creep fed either with a control diet or supplemented with glutamine or Aminogut showed longer villus than those treatments which were not creep fed (552 microns vs. 511 microns respectively) (figure 4, 5 and 6). The CFGLN-GLN treatment showed elongated, well defined and high villus (figure 7). Increased villus height could increase total luminal villus absorption area and could result in adequate digestive enzyme concentration and/or increased transport of nutrient at the villus surface. Gln has been shown to enhance epithelial repair in several models of intestinal injury and to stimulate epithelial proliferation or reduce apoptosis in cell culture (Blikslager et al., 2001). Increased uptake of Gln in the crypts not only could promote a compensatory increase in Na^+ absorption but also would place this nutrient in the ideal location to promote crypt cell production and restoration of the villus architecture**.** The CGAG-AG treatment showed deep and wide crypts. This could be explain by the fact that glutamine is donating an amide group for the biosynthesis of purine (adenine and guanine) and pyrimidine (thymine and cytosine) which are the nucleotides bases to support nucleic acid production (DNA) for rapidly dividing cells in the crypts. In RNA, the complement of adenine is uracil instead of thymine. The sow reared pigs showed what may be the perfect villi structure: healthy, well defined villus, no signs of apoptotic cells and sufficient mucin production (figure 9). Mucins are a family of high molecular weight, heavily glycosylated proteins produced by epithelial tissues (specifically by the goblet cells) in most metazoans. Two noticeable jejunal villi structure characteristics in all treatments for 28 d pigs were 1) transversal furrows that were present along the entire length of the villi and 2) the

shape of the villi were not finger-like but rather wide and tongue-like in shape. In summary, the small intestinal mucosa undergoes profound structural and developmental changes during the first 4 weeks of the pig's life and these changes are manifested in shape, size and density of the villi.

CONCLUSION

The supplementation of glutamine and glutamine plus glutamate (AminoGut) in pre- and post-weaning diets improved feed conversion, villi height, crypt depth and proliferating cells nuclei antigen (PCNA) in the first three weeks post-weaning. These findings are in the agreement with those reporting a reduction in villi atrophy when supplementing glutamine at 1% in diets during the first week post-weaning. Sow reared pigs showed the best intestinal absorptive capacity and villi architecture. More research is needed at the field level to justify the economical feasibility of adding either glutamine or AminoGut in current commercial livestock diets and the European model of weaning pigs at 28 d of age.

The existing vast knowledge of the roles of functional AA's such as glutamine and others (arginine, glutamate, proline, leucine, cysteine and tryptophan) provides the scientific basis for nutritionists to revise current nutrient requirements for livestock especially weaned pigs. These findings indicate that strong consideration must be given to Gln and glutamate as nutritionally essential amino acids for post-weaning pigs diets.

Table 1. Ingredients and Nutrients Composition of the Nursery Diets (Phase 1, 2 and 3)[a].

Ingredients, %	Phase 1	Phase 2	Phase 3
Corn	38.91	51.32	63.71
SMB 48%	20.00	25.00	30.00
Fat, CWG	3.00	3.00	3.00
Dicalcium	0.00	0.60	1.30
Limestone	0.60	0.60	0.50
Salt	0.30	0.30	0.50
L-Lysine	0.30	0.30	0.40
DL-Methionine	0.20	0.20	0.20
L-Threonine	0.15	0.15	0.18
L-Tryptophan	0.03	0.02	0.00
Granular Whey	5.00	0.00	0.00
Plasma APC	4.00	2.00	0.00
Dairy Lac 80	20.00	10.00	0.00
Fish Meal Select	6.00	5.00	0.00
ZnO	0.30	0.30	0.00
Copper Sulfate	0.05	0.05	0.05
Vitamins Mix[b]	0.01	0.01	0.01
Trace Mineral Mix[c]	0.15	0.15	0.15
Mecadox (2.5 g/lb)	1.00	1.00	0.00
Total	**100%**	**100%**	**100%**
Nutrients			
NRC ME (Mcal/kg)	3.519	3.462	3.462
Mod. ME (Mcal/kg)	3.424	3.352	3.352
CP %	21.67	21.77	20.12
TID Lysine %	1.39	1.35	1.25
TID Met %	0.52	0.50	0.45
TID M+C %	0.83	0.81	0.75
TID Threonine	0.93	0.88	0.82
TID Tryptophan	0.25	0.25	0.21
TID Isoleucine	0.77	0.78	0.73
TID Valine	0.93	0.91	0.82
Glutamate	3.69	3.79	3.62
Total P %	0.64	0.67	0.63
Available P %	0.43	0.40	0.30
Ca %	0.80	0.80	0.60
Na %	0.32	0.21	0.21
Lactose %	19.5	8.00	0.00

[a]L-Glutamine was supplemented at 1% of the diet at the expense of corn. Amino-Gut was supplemented 0.8% in phase 1 and 2; and at 0.6% in phase 3.
[b]Vitamin Mix contained per kg of premix: vitamin A Acetate 4,231,292 IU; vitamin D_3 603,174 IU; vitamin E ascorbate 24,172 IU; vitamin B_{12} 14.97 mg; riboflavin spray dried 3,023 mg; niacin 18,140 mg; d-pantothenic 12,093 mg; menadione 1,995 mg; folic acid spray dried 907 mg; biotin spray dried 121 mg.
[c]Trace mineral Mix contained: ferrous sulfate 11.02%; copper sulfate 1.10%; ethylenediamine dihydriodine 198 ppm; manganese oxide 2.64%; sodium selenite 198.4 ppm; zinc sulfate 11.02%.

Table 2. Pre-weaning Performance of No-Creep, Creep, Glutamine and AminoGut Treatments.

Variables	No-Creep	Creep	Glutamine	AminoGut	SEM	P value
No. Litters	45	45	15	15	-	-
Wean Age, d	21.5	20.5	20.3	18.9	0.134	< 0.0001
Pigs/litter	12.5	12.4	12.5	11.9	0.226	0.286
Sow Parity	3.4	3.4	3.4	3.4	0.199	0.998
Birth Wt., Kg	1.5	1.4	1.4	1.5	0.039	0.250
Wean Wt., Kg	6.5	6.4	6.4	6.5	0.261	0.980
[1]Creep Feed, g/pig	-	49.4	45.6	48.4	2.931	0.617
Mortality, pigs/litter	1.0	1.2	1.5	1.2	0.220	0.488

[1]Creep feed was offered from d 14 to d 21 thee times /d (8:00 AM, 12:00, and 4:00 PM).

Table 3. Pig Performance from week 1 to week 3 Post-weaning.

Variables	NC-CD	NC-GLN	NC-AG	CFCD-CD	CFCD-GLN	CFCD-AG	CFGLN-GLN	CFAG-AG	SEM	P value
No. Pens	6	6	6	6	6	6	6	6	-	-
No. Pigs	144	144	144	144	144	144	144	144	-	-
Initial Wt., kg	6.50	6.35	6.59	6.14	6.39	6.61	6.42	6.52	0.343	0.984
Final Wt., kg	10.4	10.20	10.66	9.51	10.06	10.08	10.65	10.34	0.256	0.128
ADG, kg/pig	0.25	0.24	0.25	0.19	0.23	0.22	0.26	0.24	0.016	0.124
Intake, kg/pig	0.33	0.28	0.30	0.27	0.27	0.28	0.29	0.29	0.021	0.644
Feed/Gain	1.35^{bc}	1.17^{ab}	1.17^{ab}	1.48^{c}	1.15^{ab}	1.30^{b}	1.11^{a}	1.21^{ab}	0.086	0.056
Mortality	0.38	1.43	0.94	1.28	0.80	0.63	0.82	0.73	0.372	0.562
Removed	1.23	0.90	2.00	2.49	1.28	1.03	1.81	0.59	0.702	0.584

NC-CD = No Creep Control Diet, NC-GLN = No Creep Glutamine, NC-AG = No Creep AminoGut, CFCD-CD = Creep Fed Control Diet-Control Diet, CFCD-GLN = Creep Fed Control Diet-Glutamine, CFCD-AG = Creep Fed Control Diet-AminoGut, CFGLN-GLN = Creep Fed Glutamine-Glutamine, CFAG-AG = Creep Fed AminoGut-AminoGut.

^{abc}***LS means within a row lacking a common superscript are different (P < 0.05).***

Table 4. Pig Performance from week 3 to week 6 post-weaning[1].

Variables	NC-CD	NC-GLN	NC-AG	CFCD-CD	CFCD-GLN	CFCD-AG	CFGLN-GLN	CFAG-AG	SEM	P value
No. Pens	6	6	6	6	6	6	6	6	-	-
Initial Wt., kg	10.45	10.32	10.72	9.44	10.18	10.10	10.78	10.43	0.426	0.457
Final Wt., kg	22.32	21.97	22.74	22.21	22.86	22.50	21.71	22.76	0.408	0.437
ADG, kg/pig	0.52	0.51	0.55	0.50	0.54	0.53	0.50	0.54	0.018	0.444
Intake, kg/pig	0.77[b]	0.74[b]	0.86[a]	0.77[b]	0.79[b]	0.79[b]	0.70[c]	0.80[b]	0.027	0.022
Feed/Gain	1.39	1.48	1.65	1.49	1.64	1.84	1.66	1.50	0.123	0.271
Mortality, %	0.01	0.00	0.35	0.13	0.16	0.01	0.19	0.01	0.127	0.434
Removed, %	1.01	1.5	1.69	1.62	0.99	1.15	1.69	1.17	0.318	0.497

[1]For the calculation of Feed Intake and FCR, the number of pens was reduced from 6 to 3 pens because two pens shared a common feeder. NC-CD = No Creep Control Diet, NC-GLN = No Creep Glutamine, NC-AG = No Creep AminoGut, CFCD-CD = Creep Fed Control Diet-Control Diet, CFCD-GLN = Creep Fed Control Diet-Glutamine, CFCD-AG = Creep Fed Control Diet-AminoGut, CFGLN-GLN = Creep Fed Glutamine-Glutamine, CFAG-AG = Creep Fed AminoGut-AminoGut.
[abc]***LS means within a row lacking a common superscript are different (P < 0.05).***

Table 5. Histology, PCNA, Intestinal Absorption Capacity and Maltase Activity of Treatments.

Variables	NC-CD	NC-GLN	NC-AG	CFCD-CD	CFCD-GLN	CFCD-AG	CFGLN-GLN	CFAG-AG	Sow Reared	SEM	P value
No. Pigs	6	6	6	6	6	6	6	6	6	-	-
Age pigs, d	28	28	28	28	28	28	28	28	28	-	-
[1]Wt., kg	6.5	6.7	6.2	5.8	6.4	6.4	6.5	6.5	6.3	0.282	0.824
Villi Height, µm	508^{ab}	515^{ab}	510^{ab}	560^{ab}	576^{a}	480^{b}	568^{a}	560^{ab}	570^{a}	23.26	0.049
Villi Width, µm	136	140	120	133	148	158	145	147	132	8.93	0.301
Crypt Dept, µm	202^{ab}	188^{b}	177^{b}	192^{ab}	218^{ab}	242^{ab}	225^{ab}	258^{a}	118^{c}	14.72	<0.0001
PCNA, cells	56^{c}	81^{a}	67^{b}	71^{ab}	86^{a}	70^{ab}	80^{a}	64^{b}	91^{a}	5.50	<0.0001
Xylose, mg/dl	14.6^{b}	13.5^{b}	11.1^{b}	14.4^{b}	9.5^{b}	14.1^{b}	15.4^{b}	13.7^{b}	42.8^{a}	1.94	<0.0001
Mannitol, mg/dl	1.8^{b}	1.5^{b}	2.3^{b}	1.9^{b}	2.8^{b}	3.0^{b}	2.8^{b}	3.0^{b}	17.7^{a}	0.677	<0.0001
Maltase, µmoles/(min. g of protein)	256.8	183.7	201.7	299.4	282.5	254.2	244.3	242.2	239.2	32.18	0.189

[1]Body weight was taken one week post-placement in the nursery. Sow reared pigs stayed with their dams for 28 d.

NC-CD = No Creep Control Diet, NC-GLN = No Creep Glutamine, NC-AG = No Creep AminoGut, CFCD-CD = Creep Fed Control Diet-Control Diet, CFCD-GLN = Creep Fed Control Diet-Glutamine, CFCD-AG = Creep Fed Control Diet-AminoGut, CFGLN-GLN = Creep Fed Glutamine-Glutamine, CFAG-AG = Creep Fed AminoGut-AminoGut. Sow Reared were pigs kept with the sow until they were 28 d of age.

[abc]***LS means within a row lacking a common superscript are different.***

Figure 1. Scanning Electron Microscopy (SEM) of jejunum of pigs No Creep Control Diet (NC-CD) treatment.

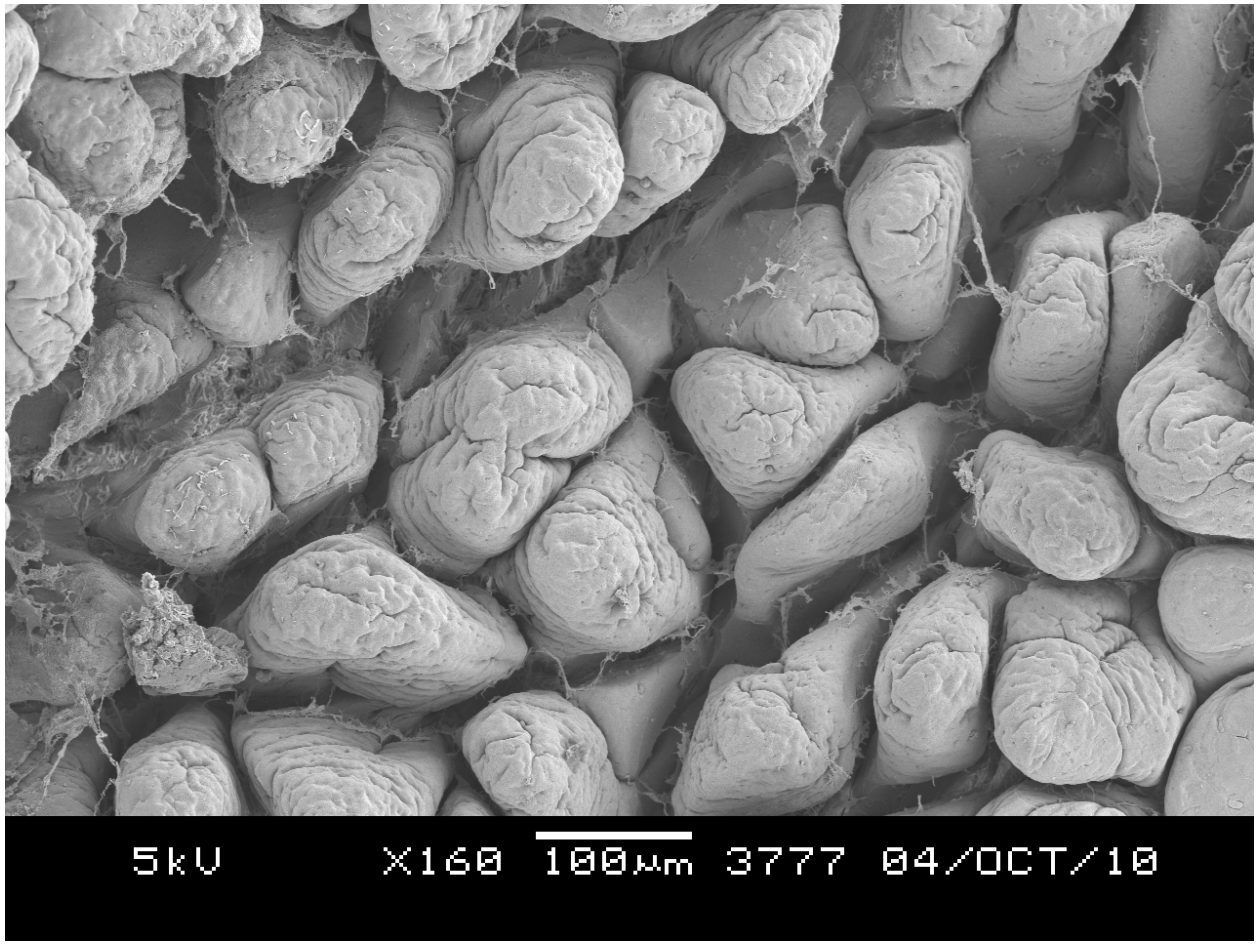

Figure 2. Scanning electron microscopy (SEM) of jejunum of pigs No Creep Glutamine (NC-GLN) treatment.

Figure 3. Scanning electron microscopy (SEM) of jejunum of pigs No Creep AminoGut (NC-AG) treatment.

Figure 4. Scanning electron microscopy (SEM) of jejunum of pigs Creep Fed Control Diet-Control Diet (CFCD-CD) treatment.

Figure 5. Scanning electron microscopy (SEM) of jejunum of pigs Creep Fed Control Diet-Glutamine (CFCD –GLN) treatment.

Figure 6. Scanning electron microscopy (SEM) of jejunum of pigs Creep Fed Control Diet-AminoGut (CFCD-AG) treatment.

Figure 7. Scanning electron microscopy (SEM) of Jejunum of pigs Creep Fed Glutamine-Glutamine (CFGLN-GLN) treatment.

Figure 8. Scanning electron microscopy (SEM) of Jejunum of pigs Creep Fed AminoGut-AminoGut (CFAG-AG) treatment.

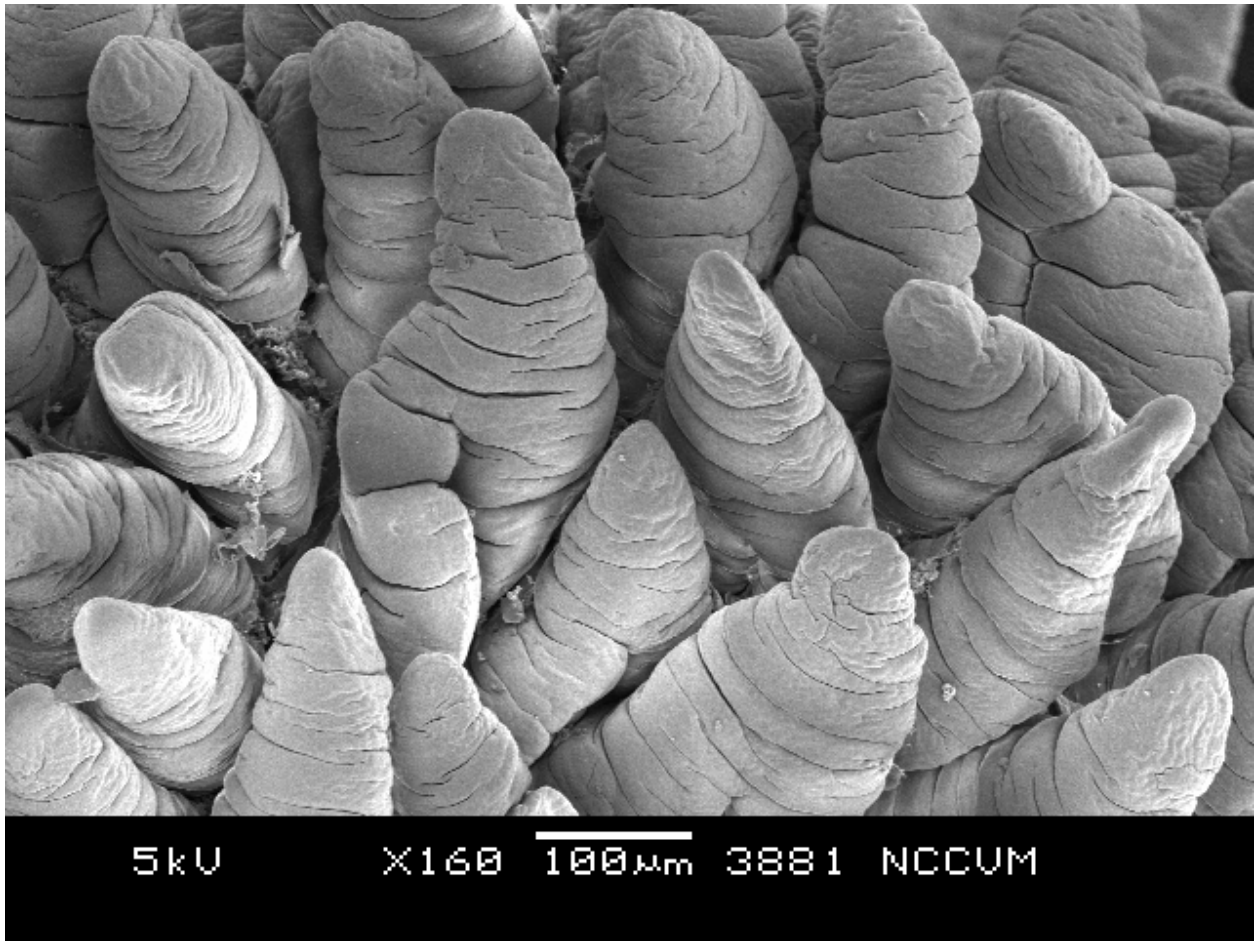

Figure 9. Scanning electron microscopy (SEM) of jejunum of 28 d old sow-reared pigs.

LITERATURE CITED

1. Alvarado, F. 1966. D-xylose active transport in the hamster small intestine. Biochim. Biophsy Acta 112:292-306.
2. Aumaitre, A and T. Corring. 1978. Development of digestive enzymes in the piglet from birth to 8 weeks. Nutr. Metab. 22:244-255.
3. Barnett, K.L., E.T. Kornegay, C.R. Risley, M.D. Lindemann and G.G. Schurig. 1989. Characterization of creep feed consumption and its subsequent effects on immune response, scouring index and performance of weanling pigs. J. Anim. Sci. 67:2698-2708.
4. Bikker, P., A. Dirkzwager, J. Fledderus, P. Trevisi, I. le Huerou-Luron, J.P. Lalles and A. Awati. 2006. The effect of dietary protein and fermentable carbohydrates levels on growth performance and intestinal characteristics in newly weaned piglets. J. Anim. Sci. 84:3337-3345.
5. Blikslager, A., E. Hunt, R. Guerrant, M. Rhoads and R. Argenzio. 2001. Glutamine transporter in crypts compensates for loss of villus absorption in bovine cryptosporidiosis. Am. J. Physiol. Gastrointest. Liver Physiol. 281:G645-G653.
6. Boutry, C., C. Bos, H. Matsumoto, P. Even, D. Azzout-Marniche, D. Tome and F. Blachier. 2011. Effects of monosodium glutamate supplementation on glutamine metabolism in rats. Front. Biosci. E3:279-290.
7. Burger, A., J.O. Henck, S. Hetz, J.M. Rollinger, A.A. Weissnicht, and H. Stottner. 2000. Energy/temperature diagram and compression behavior of the polymorphs of D-mannitol. J. Pharm. Sci. 89:457-468.

8. Burrin, D.G and P.J. Reeds. 1997. Alternative fuels in the gastrointestinal tract. Curr. Opin. Gastroenterol. 13:165-170.
9. Cera, K.R., D.C. Mahan, R.F. Cross, G.A. Reinhart, and R.E. Whitmoyer. 1988a. Effect of age, weaning and post-weaning diet on small intestinal growth and jejunal morphology in young swine. J. Anim. Sci. 66:574.
10. Cera, K. R., D.C. Mahan and G.A. Reinhart. 1990. Effect of weaning, week postweaning and diet composition on pancreatic and small intestinal luminal lipase response in young swine. J. Anim. Sci. 68:384-391.
11. Chamorro, S., C. de Blas, G. Grant, I. Badiola, D. Menoyo and R. Carabano. 2010. Effect of dietary supplementation with glutamine and a combination of glutamine-arginine on intestinal health in twenty-five-day-old weaned rabbits. J. Anim. Sci. 88:170-180.
12. Chang, R.W., P. J. David, Jung-Tak Oh, S. Andreoli, H.B. Kim, D. Fauza and T. Jaksic. 2006. Serial Transverse Enteroplasty enhances intestinal function in a model of short bowel syndrome. Ann. Surg. 243 (2):223-228.
13. Clifford, A. 2006. Nutrition-based health in animal production. Nutr. Res. Rev. 19:79-89.
14. Dahlqvist, A. 1984. Assay of intestinal disaccharidases. Scand. J. Clin. Lab. Invest. 44:169-172.
15. Doerfler, R.E., L.D. Cain, F.W. Edens, C.R. Parkhurst, M.A. Qureshi and G.B. Havenstein. 2000. D-Xylose absorption as a measurement of malabsorption in poult enteritis and mortality syndrome. Poultry Sci. 79:656-660.

16. Eberts, T.J., R.H.B. Sample, M.R. Glick and G.H. Ellis. 1979. A simplified, colorimetric micro method for xylose in serum or urine, with phloroglucinol. Clin. Chem. 25:1440-1443.
17. Eggleston, G. and W. Harper. 2006. Determination of sugarcane deterioration at the factory: Development of a rapid, easy and inexpensive enzymatic method to measure mannitol. Food Chemistry 98: 366-372.
18. Goodwin, M.A., K.S. Latimer and O.J. Fletcher. 1984a. Quantification of intestinal D-xylose absorption in normal turkeys. Poultry Sci. 63:1742-1747.
19. Goodwin, M.A., K.S. Latimer, and B.N. Nersessian. 1984b. Quantification of intestinal D-xylose absorption in normal and reovirus-inoculated turkeys. Avian Dis. 28:959-967.
20. Graefe, H., B. Gütschov, H. Gerhing and L. Dibbelt. 2003. Sensitive and specific photometric determination of mannitol in human serum. Clinical Chemistry and Laboratory Medicine 48 (8): 1049-1055.
21. Häussinger, D., F. Lang and W. Gerok. 1994. Regulation of cell function by the cellular hydration state. Am. J. Physiol. 267:E343-E355.
22. Haynes, T.E., P. Li, X. Li, K. Shimotori and H. Sato. 2009. L-Glutamine or L-alanyl-L-glutamine prevents oxidant or endotoxin-induced death of neonatal enterocytes. Amino Acids 37:131-142.
23. James, L.A., P.G. Lunn and M. Elia. 1998. Glutamine metabolism in the gastrointestinal tract of the rat assessed by the relative activities of Glutaminase and Glutamine Synthetase. Br. J. Nutr. 79:365-372.

24. Jennings, D.B., M. Ehenshaft, D.M. Parr and J.D. Williamson. 1998. Roles for mannitol and mannitol Dehydrogenase in active oxygen-mediated plant defense. Proc. Natl. Acad. Sci. 95:15129-15133.
25. Jiang, Z.Y., L.H. Sun, Y.C. Lin, X. Y. Ma, C.T. Zheng, G.L. Zhou, F. Chen and S.T. Zou. 2009. Effects of dietary glycyl-glutamine on growth performance, small intestinal integrity, and immune responses of weaning piglets challenged with lipopolysaccharide. J. Anim. Sci. 87:4050-4056.
26. Johnson, S.A., I.G. van Tets and S.W. Nicolson. 1999. Sugar preferences and xylose metabolism of a mammal pollinator, the Namaqua Rock mouse (*Aethomys namaquensis*). Physiological and Biochemical Zoology 72(4):438-444.
27. Kenworthy, R. and W.E. Crabb. 1963. The intestinal flora of young pigs with particular reference to early weaning, E. Coli and scours. J. Comp. Path. 73:215-228.
28. Kidder, D.E. and M.J. Manners. 1978. Carbohydrases in the small intestine mucosa of sow-reared and 3-wk weaned pigs. Proc. Nutr. Soc. 37 (2): 51A.
29. Kidder, D.E. and M.J. Manners. 1980. The level of distribution of carbohydrases in the small intestine mucosa of pigs from 3 weeks of age to maturity. Br. J. Nutr.4 (1): 141-153.
30. Kim, S.W and G. Wu. 2009. Regulatory role of amino acids in mammary gland growth and milk synthesis. Amino acids 37:89-95.
31. Klasing, K.C. 2007. Nutrition and the immune system. British Poultry Sci. 48:525-537.

32. Laker, M.F., H.J. Bull, and I.S. Menzies. 1982. Evaluation of mannitol for use as a probe marker of gastrointestinal permeability in man. Eur. J. of Clinical Investigation 12:485-491.

33. Le, A.S. and K.B. Mulderrig. 2001. Sorbitol and mannitol. In: Nabors OL, editor. Alternative sweeteners, 3rd edition. Marcel Dekker Inc. New York, NY.

34. Le Boucher, J., C. Coudray-Lucas, E. Lasnier, A. Jardel, O.G. Ekindjian and L.A. Cynober. 1997. Enteral administration of ornithine-ketoglutarate or arginine-ketoglutarate: A comparative study of their effects on glutamine pools in burn-injured rats. Crit. Care Med. 25:293-298.

35. Li, D.F., J.L. Nelssen, P.G. Reddy, F. Blecha, R. Klemm and R.D. Goodband. 1991. Interrelationship between hypersensitivity to soybean proteins and growth performance in early-weaned pigs. J. Anim. Sci. 69:4062-4069.

36. Li, P., Y.L. Yin, D.F. Li, S.W. Kim and G. Wu. 2007. Amino acids and immune function. Br. J. Nutr. 98:237-252.

37. Liu, T., J. Peng, Y. Xiong, S. Zhou and X. Cheng. 2001. Effects of dietary glutamine and glutamate supplementation on small intestine structure, active absorption and DNA, RNA concentrations in skeletal muscle tissue of weaned piglets during d 28 to 42 d of age. Asian-Aust. J. Anim. Sci. vol. 15, No. 2:238-242.

38. Longland, A.C. 1991. Digestive enzyme activities in pigs and poultry. In: M.F. Fuller (Ed.) In vitro digestion for pigs and poultry. Pp 5-6. C.A.B. International, Wallingford, U.K.

39. Mahan, D.C. and E.A. Newton. 1993. Evaluation of feed grains with dried skim milk and added carbohydrate sources on weanling pig performance. J. Anim. Sci. 71:3376-3382.

40. Mansoori, B., H. Nodeh, M. Modirsanei, S. Rahbari and P. Aparnak. 2008. D-xylose absorption test: A tool for the assessment of the effect of anticoccidials on the intestinal absorptive capacity of broilers during experimental coccidiosis. Anim. Feed Sci. Technol. Doi: 10.1016.

41. McDowell, E.M. and B.F. Trump. 1976. Histological fixatives suitable for diagnostic light and electron microscopy. Arch. Pathol. Lab. Med. 100:405.

42. Meijer, G.A., J. van der Meulen and A.M. Van Vuuren. 1993. Glutamine is a potentially limiting amino acid for milk production in dairy cows: A hypothesis. Metabolism 42:358-364.

43. Menzies, I.S. 1984. Transmucosal passage of inert molecules in health and disease. In E. Skadhauge and K. Heintze (Ed.) Intestinal absorption and secretion. Pp 527-543. MTP Press, Lancaster, U.K.

44. Miller, B.G., T.J. Newby, C.R. Stokes and F.J. Bourne. 1984. Influence of diet on post-weaning malabsorption and diarrhea in the pig. Res. Vet. Sci. 36:187.

45. Nappert, G., G.A. Zello and J.M. Naylor. 1997. Intestinal metabolism of glutamine and potential use of glutamine as a therapeutic agent in diarrheic calves. J. Am. Vet. Med. Assoc. 211:547-553.

46. Nasrallah, S.M. and F.L. Iber. 1969. Mannitol absorption and metabolism in man. Am. J. Med. Sci. 258 (2): 80-88.

47. Newby, T.J., B.G. Miller, C.R. Stokes and F.J. Bourne. 1983. Hypersensitivity to dietary antigens as the predisposing factor in post-weaning diarrhea. Pig Vet. Soc. Proc. 10:50.

48. Newsholme, P. 2001. Why is L-glutamine metabolism important to cells of the immune system in health, post injury, surgery or infection? J. Nutr. 131:2515S-2522S.

49. Palmer, N.C. and T. J. Hulland. 1965. Factors predisposing to the development of coliform gastroenteritis in weaned pigs. Can. Vet. J. 6:310-316.

50. Plunske, J.R., D.J. Hampson and I.H. Williams. 1997. Factors influencing the structure and function of the small intestine in the weaned pig. A review. Livest. Prod. Sci. 51:215-236.

51. Poulton, A. and M.H. Winterborn. 1987. Arch. Dis. Child 62(7):729-731.

52. Radberg, K., M. Biernat, A. Linderoth, R. Zabielski, S.G. Pierzynowski and B.R. Westrom. 2001. Enteral exposure to crude red kidney bean lectin induces maturation of the gut in suckling pigs. J. Anim. Sci. 79:2669-2678.

53. Reeds, P.J., D.G. Burrin, B. Stoll, F. Jahoor, L. Wykes, J. Henry and M.E. Frazer. 1997. Enteral glutamate is the preferential source for mucosal glutathione synthesis in fed piglets. Am. J. Physiol. 273:E408-E415.

54. Reeds, P.J., D.G. Burrin, B. Stoll and F. Jahoor. 2000. Intestinal Glutamate Metabolism. J. Nutr. 130:978S-982S.

55. Rhoads, J.M. and G. Wu. 2009. Glutamine, arginine, and leucine signaling in the intestine. Amino acids 37:111-122.

56. Salem, A.A., A.e. Cocco and T.R. Hendrix. 1965. Inhibition of absorption of D-xylose by phlorizin in the anesthetized rat. Am. J. Physiol. 204:165-168.

57. Schock, H. and L. Goldstein. 1981. Inter-organ relationships for glutamine metabolism in normal and acidotic rats. Am. J. Physiol. 240:E519-E525.

58. Shen, B., S. Hohmann, R.G. Jensen, and A.H. Bohnert. 1999. Roles of sugar alcohols in osmotic stress adaption. Replacement of glycerol by mannitol and sorbitol in yeast. Plant Physiol. 121:45-52.

59. Skrzypek, T., J.L. Valverde, H. Skrzypek, J. Wolinski, W. Kazimierczak, S. Szymanczyk, M. Pawlowska and R. Zabielski. 2005. Light and scanning electron microscopy evaluation of the postnatal small intestinal mucosa development in pigs. J. Physiology and Pharmacology. 56:71-87.

60. Souba, W.W., K. Herskowitz, R.M. Salloum, M.K. Chena and T.R. Augsten. 1990. Gut glutamine metabolism. J. Parenter. Enter. Nutr. 14 (Suppl. 4):45S-50S.

61. Stoll, B and D.G Burrin. 2006. Measuring splanchnic amino acid metabolism in vivo using stable isotopic tracers. J. Anim. Sci. 84:E60-E72.

62. Thomsson, A., D. Rantzer, B.R. Westrom, S.G. Pierzynowski and J. Svendsen. 2007. Effects of crude red kidney bean lectin (phytohemagglutinin) exposure on performance, health, feeding behavior, and gut maturation of pigs at weaning. J. Anim. Sci. 85:477-485.

63. Trottier, N.L., C.F. Shipley and R.A. Easter. 1997. Plasma amino acid uptake by the mammary gland of the lactating sow. J. Anim. Sci. 75:1266-1278.

64. Van de Poll, M.C., P.B. Soeters, N.E. Deutz, K.C. Fearon and C.H. Dejong. 2004. Renal metabolism of amino acids: Its role in interogan amino acid exchange. Am. J. Clin. Nutr. 79:185-197.

65. Van der Hulst, R.R.W.J., B.K. Van Kreel, M.F. Von Meyenfeldt, R.J. Brummer, J.W. Arends, N.E. Deutz and P.B. Soeters. 1993. Glutamine and the preservation of gut integrity. Lancet 431:1363-1365.

66. Wang, J.J., X. Chen, P. Li, X.L. Li, H.J. Zhou, F.L. Wang, D.F Li, Y.L. Yin and G. Wu. 2008a. Gene expression is altered in piglet small intestine by weaning and dietary glutamine supplementation. J. Nutr. 138:1025-1032.

67. Windmueller, H.G. and A.E. Spaeth. 1975. Intestinal metabolism of glutamine and glutamate from the lumen when compared to glutamine from the blood. Arch. Biochem. Biophys. 171:662-672.

68. Windmueller, H.G. and A.E. Spaeth. 1976. Metabolism of absorbed aspartate, asparagine and arginine by rat small intestine in vivo. Arch. Biochem. Biophys. 175:670-676.

69. Wu, G. A.G. Borbolla and D.A. Knabe. 1994a. The uptake of glutamine and release of arginine, citrulline and proline by the small intestine of developing pigs. J. Nutr. 124:2437-2444.

70. Wu, G., D.A. Knabe and N.E. Flynn. 1994b. Synthesis of citrulline from glutamine in pig enterocytes. Biochem. J. 299:115-121.

71. Wu, G., S.A. Meier and D.A. Knabe. 1996. Dietary glutamine supplementation prevents jejunal atrophy in waned pigs. J. Nutr. 126:2578-2584.

72. Wu, G., T.L. Ott, D.A. Knabe and F.W. Bazer. 1999. Amino acid composition of the fetal pig. J. Nutr. 129:1031-1038.

73. Wu, G. 2009. Amino acids: metabolism, functions, and nutrition. Amino acids 37:1-17.

74. Wu, G. 2010. Recent Advances in Swine Amino Acid Nutrition. J. Anim. Sci. Biotech. 1(2):118-130.

75. Wu, G. 2010a. Recent Advances in Swine Amino Acid Nutrition. J. Anim. Sci. Biotech. 1:49-61.

76. Wu, G. 2010b. Functional amino acids in growth, reproduction and health. Adv. Nutr. 1:31-37.

77. Yi, G.F., J.A. Carroll, G.L. Allee, A.M. Gaines, D.C. Kendall, J.U Usry, Y. Toride, and S. Izuru. 2005. Effect of glutamine and spray-dried plasma on growth performance, small intestine morphology, and immune responses of Escherichia coli $K88^{+}$-challenged weaned pigs. J. Anim. Sci. 83:634-643.

78. Yin, Y.L., R.L. Huang, T.J. Li, Z. Ruan, M.Y. Xie, Z.Y. Deng, Y.Q. Hou and G. Wu. 2010. Amino acid metabolism in the portal-drained viscera of young pigs: effects of dietary supplementation with chitosan and pea hull. Amino acids 39:1581-1587.

79. You-Sheng, L., L. Jie-Shou, L. Ninj, J. Zhi-Wei, Z. Yun-Zhao, L. Nan-Yun and L. Fang-Nan. 1998. Evaluation of various solutions for small bowel graft preservation. WJG 4 (2): 140-143.

80. Ziegler, T.R., M.Evans, C. Estavirez, and D.P. Jones. 2003. Trophic and cytoprotective nutrition for intestinal adaptation, mucosal repair and barrier function. Annu. Rev. Nutr. 23:229-261.

81. Zilva, J.F. and P.R Pannall. 1984. Clinical chemistry in diagnosis and treatment. 4th ed. Lloyd-Luke, London.

CHAPTER 6

CONCLUDING THOUGHTS

Experiment 1: The influence of maternal age on the immune competence of her progeny is known to be important but has been more extensively studied in dairy cattle than pigs. The most important and unexpected, finding of this trial was that the longer the sow nursed the litter, the better her progeny tended to perform in ADG, gain: feed, viability (mortality plus illness), loin and fat depth. Another important finding was the failure of the **2W** pigs to completely retain the substantial weaning weight advantage over the lighter **SR** and **14W** pigs throughout the post-weaning period. The undeniable fact of this experiment is that long-term whole-body and carcass growth were benefited by piglet spending 20 d with the sow when compared to either 14 or 2 d. The influence of the sow beyond the colostrum period and even to 14 d of age is significant. The biological basis for the far reaching effects of the sow, on the performance of her progeny is not clear. In this study, we failed to characterize the health status (cortisol, acute-phase proteins, T-helper cells (CD4+) and cytotoxic T-cells (CD8+)) of the pigs at weaning in order to understand their post-weaning growth. By doing that, it would have given us a clear picture of their ability to counteract an immune challenge post-weaning. We hypothesize that the pigs weaned at 2 d of age (**2W**) may have had a compromised immune system which in turn was expressed by higher mortality and slower post-weaning growth when compared to the **SR** and **14W**. In order for the animal to mount a vigorous immune response, the immune system can account for about 9% of all nutrient use and more specifically 3% of the net energy (Klasing, 2007). Humphrey (2010) argued that this change in metabolism is the result of increased expression of nutrient

transporters for lysine, arginine and glucose in immune tissues. In the other hand, skeletal muscle is being catabolized to release amino acids for the liver to use in the synthesis of acute-phase proteins (Reeds et al., 1994). We propose that the sow influences her progeny's performance beyond the colostrum period and in a profound way. Based on what is known in human nutrition, the maternal influence of milk is important to the development of immune competence beyond the contribution of colostrum. We hypothesize that **SR** and **14W** pigs could have had a more balanced immune system (establishment of commensal microbiota, in the gastrointestinal tract, an innate immune response that does not cause excessive tissue damage or nutrient repartitioning and adaptive immunity with proper lymphocyte differentiation between T-helper cell subsets) when compared to the **2W** pigs. By balancing the immune system, an animal can quickly and efficiently overcome health challenges that would otherwise decrease its productivity. Immune balance can be achieved through nutrition, especially as the nutrient requirements of the immune system are better characterized.

Experiment 2: The most significant finding of this study was that sow parity, sow colostral IgG concentration and birth order only explained 13% of the total variability found in pig IgG concentration. We hypothesized that these factors would have accounted for a greater percentage of the total variability found in pig IgG concentration. Another significant finding was that, contrary to what we would have expected, birth weight had no effect on pig IgG concentration. This lack of effect could be explained by the fact that we physically aided each new born piglet to suckle from their dam. Piglet IgG concentration and birth weight had the greatest effect of any of the variables measured on survival percent at weaning. In

retrospective, we thought that absorption and uptake of macromolecules (intestinal closure) occur between 2-3 d post-birth. After we conducted our third experiment (IgG time course absorption), we now know that intestinal closure occur in the first 24 h post-birth therefore we would have determined the IgG content of those piglets in the first 24 h post-birth. The content of IgG in the colostrum decreased rapidly from 95.6% at 0 h post-birth to 14.2% at 24 h post-birth (Klobasa et al., 1987).

Experiment 3: Colostrum itself plays a most important role in the induction of intestinal closure. The formation of colostrum in the sow starts about one month prepartum with an intensive transfer of immunoglobulins from the serum to the udder (Jonsson, 1973). This process virtually ceases after parturition resulting in a sharp decrease of the IgG concentration in the colostrum (Frenyo, 1980; Klobasa, 1986). These authors also confirmed that IgG absorption by the piglet starts declining 24 h postpartum which was confirmed by us as we analyzed the FcRn gene expression in the first 24 h post-birth. We saw a down regulation of this receptor after 12 h post-birth. Blecha (1998) indicated that the IgG concentration of colostrum is highest during the birth process and decreases during the first day of lactation. We were unable to detect any IgG which may have passed from the placenta to the fetus immediately after birth. These findings confirm that neonatal piglets at birth possess almost no serum immunoglobulins and undetectable levels of specific antibodies. It took more than one h to detect any IgG in piglet serum for animals gavaged at birth and more than 2 h for the pigs gavaged at 12 h of age, regardless of whether they were fed or fasted. Another interesting finding of this study was that the greatest absorption of IgG will occur around 8 h after administration and start leveling off at 12 h. This emphasizes the fact that if

there is an intention to administer a colostrum replacement supplement for late-born pigs, gilt progenies and in general small pigs, the best time to do that is during the first day post-birth.

Experiment 4: The supplementation of glutamine and glutamine plus glutamate (AminoGut) in pre- and post-weaning diets improved feed conversion, villi height, crypt depth and proliferating cells nuclei antigen (PCNA) in the first three weeks post-weaning. These findings are in the agreement with those reporting a reduction in villi atrophy when supplementing glutamine at 1% in diets during the first week post-weaning. Sow reared pigs showed the best intestinal absorptive capacity and villi architecture. More research is needed at the field level to justify the economical feasibility (Return over Feed and Margin per Pig) of adding either glutamine or AminoGut in current commercial livestock diets. We also argue that more consideration needs to be given to extending the length of lactation days in commercial swine units given we learned in this study with the villi arquitecture of 28 d sow reared pigs. The role of Glutamine as the main fuel (over glucose) for intestinal cells proliferation has been extensively studied (Wu et al., 1994a). The contribution of its amide group for the synthesis of nucleotides such as Purines and Pyrimidines makes Glutamine an ideal amino acid for supplementation in post-weaning diets. The existing vast knowledge of the roles of functional AA's such as glutamine and others (arginine, glutamate, proline, leucine, cysteine and tryptophan) provides the scientific basis for nutritionists to revise current nutrient requirements for livestock especially weaned pigs. These findings indicate that strong consideration must be given to Gln and glutamate as nutritionally essential amino acids for post-weaning pigs diets.

LITERATURE CITED

1. Blecha, F. 1998. Immunological aspects: comparison with other species. In: Verstegen, M.W.A., Moughan, P.J., Schama, J.W. (eds.), the lactating sow. Wageningen Pers, Wageningen, pp.23-44.
2. Frenyo, V.L., G. Pethes, T. Antal and I. Szabo. 1980. Changes in colostral and serum IgG content in swine in relation to time. Vet. Res. Comm. 4: 275-282.
3. Humphrey, B.D. 2010. Immunity lessons and actions: Practical Implications. J. Appl. Poult. Res. 19:174-181.
4. Jonsson, A. 1973. Transfer of immunoglobulins from mother to offspring in the pig. Acta vet. Scand. Suppl. 43:1-64.
5. Klasing, K.C. 2007. Nutrition and the immune system. Br. Poult. Sci. 48(5):525-537.
6. Klobasa, F. J.E. Butler, E. Werhahn and F. Habe. 1986. Maternal-neonatal immunoregulation in swine: Influence of multiparity on the novo immunoglobulin synthesis by piglets. Vet. Immunol. Immunopathol. 11:149-159.
7. Klobasa, F., E. Werhahn and J.E. Butler. 1987. Composition of sow milk during lactation. J. Anim. Sci. 64:1458-1466.
8. Reeds, P.J., C.R. Fjeld and F. Jahoor. 1994. Do the differences between amino acids compositions of acute-phase and muscle proteins have a bearing on nitrogen loss in traumatic states? J. Nutr. 124:906-910.
9. Wu, G. A.G. Borbolla and D.A. Knabe. 1994a. The uptake of glutamine and release of arginine, citrulline and proline by the small intestine of developing pigs. J. Nutr. 124:2437-2444.