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The effects of gestational folic acid supplement of sows on offspring immune organ and muscle development and postnatal immune and growth response

by

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A dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of
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This is to certify that the Doctoral dissertation of

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For the Graduate College

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CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

Dissertation Organization

This dissertation has been organized into: 1) an introductory chapter, which describes the general concepts of the research conducted and previous research conducted in this area; 2) two research papers that were prepared for publication in an appropriate scientific journal; and 3) a general discussion chapter that summarizes the major conclusions of the research conducted.

Introduction

An economically important goal in swine production is the production of healthy, viable offspring, which grow muscle tissue rapidly and produce highly muscled carcasses. Numerous environmental factors, including the dam's nutritional status, influence whether the offspring will survive and what proportion of the offspring's genetic capacity for growth will be realized both pre- and postnatally. It has been demonstrated that by affecting prenatal growth (expressed as DNA accretion), postnatal muscle content (Miller et al., 1975), immune capacity (Chandra, 1992), and survival (Pond and Wu, 1981) can be affected. One key mechanism for increased prenatal growth is thought to be alleviation of any limitation in DNA synthesis.

General Fetal Development

Conceptus growth, defined as growth of uterine, placental, and fetal tissues, is characterized by rapid expansion of the placenta early in pregnancy. In swine, placental

weight increases approximately 200 g from conception to day 65 post conception, but only an additional 50 grams by day 100 post conception (Knight et al., 1977).

Fetal growth primarily occurs via hyperplasia in early to mid pregnancy. Total fetal DNA accretion rate averages only 0.385 mg/day from conception to day 30 (Tremblay et al., 1989). Fetal DNA accretion during the period between day 50, derived from measurements of total fetal DNA content (Matte et al., 1993), and day 63, estimated from fetal liver, kidney, and cerebrum DNA content (Schoknecht et al., 1994), fetal muscle DNA concentration (Hakkarainen, 1975) and an estimation of the remaining tissue DNA content, averages 47.2 mg/day.

The sources of precursors necessary for fetal DNA synthesis have not been studied in swine. It has been demonstrated that in rats on gestation day 20 to 21, transplacental passage of nucleotides, nucleosides, and bases is possible (Hayashi and Garvey, 1968). Numerous structural differences exist in the placenta of swine versus rodents. The placenta of the pig contains six layers of maternal and fetal membranes, decreasing its permeability to various substances. The placenta of rats, mice, and humans all contain only three layers of membranes, increasing the permeability. Comparison of the exchange efficiency of oxygen across the placenta of the horse, cow, pig, Rhesus monkey, guinea pig, and rabbit demonstrated that the pig has the lowest exchange efficiency (Schroder, 1982). It is therefore hypothesized that placental transfer of the precursor nucleotides, nucleosides, or bases necessary for DNA synthesis is minimal in swine and therefore these substances must be synthesized in the fetus.

The substrates necessary for DNA synthesis within the fetus can be supplied either

via degradation of existing nucleic acids and salvage of the degradation products or *de novo* synthesis of the individual nucleotide bases. The salvage pathway is not sufficient to fulfill the needs for purine synthesis in the mouse embryo (Alexiou and Leese, 1992). It is also hypothesized that the pool of thymidine present in the gamete is inadequate for the salvage pathway to be the sole supply (O'Neil, 1998).

General Fetal Muscle Development

The potential economic benefits from increased muscle development in the prenatal pig include fewer days to market, reduced feed required per unit of body growth, and increased carcass leanness. The number of muscle cells, or fibers, in swine is determined by the amount of fetal hyperplasic growth which occurs from conception thru day 85 to 90 of gestation (Wigmore and Stickland, 1983a). Subsequent muscle growth is primarily due to hypertrophy and results from increased protein synthesis in each muscle cell or fiber. The primary source of DNA for postnatal muscle growth is the muscle satellite cell (Campion, 1984). Unlike muscle fiber cells, satellite cells are capable of mitotically dividing postnatally. Satellite cells then fuse with existing muscle fibers, resulting in a postnatal increase in DNA.

Fetal Myogenesis

Fetal muscle growth involves two major events: myoblast proliferation to increase cell number, and the formation of multinucleated muscle cells, referred to as muscle fibers. Early in gestation, mesenchymal cells first differentiate into committed myogenic precursor cells. These myogenic precursor cells (myoblasts) continue to proliferate though early

gestation until the myoblasts align and eventually fuse. The result of this fusion is an elongated, multinucleated myotube that synthesizes muscle proteins but does not divide. Early in this stage of muscle development, nuclei of the myotube are located centrally within the fiber. As myotube synthesis of muscle proteins continues, the nuclei are displaced to the periphery, at which point the cells are referred to as "primary muscle fibers". Primary muscle fibers serve as the structural framework upon which additional fibers will form.

In addition to the early myoblast migration described above which results in primary fiber formation, additional myoblasts migrate, proliferate, and fuse later in gestation. These later forming secondary myotubes align with existing primary fibers, resulting in the characteristic muscle structure in which a small number of primary fibers are located at the center of the muscle and are surrounded by a larger number of secondary fibers. A third type of myoblast present in the fetus is the satellite cell. Satellite cells diverge from the late myoblast lineage and do not fuse with the muscle fiber at this time. Satellite cells serve as the source of DNA postnatally, either during growth or injury repair.

Formation of muscle fibers generally occurs during mid-gestation, but specific developmental patterns of particular muscles may vary. For example, at 60 days of gestation, moderate growth rates of 100 g/d/1,000 g dry muscle, are observed in the masseter and forelimb muscles (supraspinatus, triceps, and extensor carpi radialis), high growth rates, 150 to 200 g/d/1,000 g dry muscle, in the hind limb muscles (biceps femoris, gastrocnemius group and peroneus longus) and a low growth rate, 57 g/d/1,000 dry muscle, in the longissimus dorsi (Swatland, 1973). By day 90 of gestation growth rates of all muscles have declined, although the reduction in rate is greater in the hind limb muscles than longissimus,

57 versus 12% (Swatland, 1973). The only muscle that exhibited a marked increase, 54%, in growth rate postnatally is the longissimus dorsi (Swatland, 1973).

Late in gestation the rate of myoblast proliferation and differentiation slows to the point that the number of muscle fibers is fixed at birth. In swine, the fiber number is determined by approximately day 90 of gestation (Wigmore and Stickland, 1983a). Myogenic cells which have not fused with muscle fibers by this time, satellite cells, retain the ability to divide postnatally.

Effect of Prenatal Muscle Growth on Performance

Fetal muscle development can impact the growth, weight, and carcass muscle content and quality postnatally in swine. Wigmore and Stickland (1983b) demonstrated that the quantity of fetal pig muscle DNA, RNA, and protein influence birth weight of swine. But, the impact of prenatal development is not limited to the neonatal period. Total muscle fiber number at birth is positively correlated to pig weight on days 0, 112, and 154 postpartum, and muscle mass (Miller et al., 1975). It is, therefore, hypothesized that by increasing fetal muscle DNA content and muscle fiber number at birth, postnatal muscle growth, and carcass muscle percentage will be increased. Comparison of small (939 g) and large (2085 g) littermate pigs at birth demonstrate that small pigs have a 16% reduction in slaughter weight and a 10% reduction in total fiber number of the semitendinosus muscle when slaughtered at a common day of age (Handel and Stickland, 1988).

Since mitotic division does not occur after fetal fusion of myofibers, it can be concluded that to increase the amount of muscle DNA at birth, an increase in the number of

myoblasts prior to fusion must occur. Coutinho et al. (1993) observed that rapidly growing, heavily muscled quail undergo a delay in muscle cell differentiation, allowing muscle precursor cells to proliferate for a longer time. This delay resulted in an increase in the pool of myogenic cells available. A similar delay in myogenic cell differentiation and resultant increase in production of myotubes is observed in double muscled cattle (Quinn et al., 1990). This increase in fiber number in double-muscled cattle is associated with a lack of myostatin (Bass et al., 1999). These experiments are evidence that an increase in myogenic cells can result in an increase in muscle content of the offspring.

Muscle Fiber Types

Numerous methods of classifying muscle fibers exist. Two of the most popular methods are classification based on the muscle fiber color (red, intermediate, and white) and contractile speed (slow, intermediate, and fast, respectively). The color of a muscle fiber is indicative of the proportion of the red versus white or intermediate fibers contained within. The intensity of the color within the fiber is a function of the myoglobin content. Table 1 summarizes the differences between fiber types using these classification methods.

In swine, red-type muscle fibers are located at the center of a bundle of fibers and are surrounded by white and intermediate-type muscle fibers (Davies, 1972). Due to this characteristic organization, red fibers are thought to originate almost exclusively from primary fibers while secondary fibers result in production of intermediate and white fibers.

Table 1. Characteristics of red, intermediate, and white muscle fiber types.

	Fiber type		
Characteristics	Red	Intermediate	White
Color	Red	Red	White
Myoglobin content	High	High	Low
Fiber diameter	Small	Small-Intermediate	Large
Contraction speed	Slow	Fast	Fast
Capillary Density	High	Intermediate	Low
Oxidative Metabolism	High	Intermediate	Low
Glycolytic Metabolism	Low	Intermediate	High
Fiber diameter	Small	Intermediate	Large

(Adapted from Forrest et al., 1975)

Overview of the Immune System Development

While increased fetal muscle fiber number has the potential to increase carcass muscle content at slaughter, enhanced capacity of the neonate immune system has the potential to increase survival and growth performance of the offspring from birth to market. The capability of the neonatal immune system to respond to an immune challenge is determined by the number and type of cells present and the functionality of each of the cells.

Animals react to an immune challenge via an innate and/or adaptive immune response. Both innate and adaptive immunity are dependent on particular types of white blood cells (leukocytes) present. An innate immune response is critical in the early phases of an immune response and is mediated largely by nonspecific granulocytes. These granulocytes are phagocytic cells that engulf and destroy antigens. In contrast, an adaptive

immune response is mediated by lymphocytes which act in response to specific antigen and can result in lifelong protection to re-infection with the same antigen.

All cellular elements of the immune system are derived ultimately from the same progenitor cells, hematopoietic stem cells, located in the bone marrow of mammals. The two main categories of leukocytes that result from the hematopoietic stem cells are the myeloid progenitor and common lymphoid progenitor cells. These progenitor cells further differentiate into precursors to all cell types necessary to support both an innate and adaptive immune response.

Development of the Cellular Components of the Immune System

Myeloid progenitor cells differentiate into macrophages and granulocytes of the immune system. Macrophages play a critical role in innate immunity due to their action of engulfing and digesting antigens. Immature macrophages, referred to as monocytes, circulate in the blood and differentiate continuously upon migration into tissue.

Granulocytes, also originating from the myeloid progenitor cells, possess densely stained cytoplasmic granules. The three types of granulocytes present in swine are: neutrophils, which are involved in the innate immune response, eosinophils, which act primarily in the defense against parasitic infections, and basophils/mast cells, which play a role in protection of the mucosal surfaces of the body and release histamine in allergic responses.

The common lymphoid progenitor cells give rise to B and T lymphocytes. Upon activation, B lymphocytes differentiate into plasma cells, which secrete antibodies, and

memory cells, which are responsible for the rapid response in a secondary infection with the same antigen. T lymphocytes can be further categorized into cytotoxic T cells, primarily involved in elimination of viral antigens from the body, and helper T cells, involved in activation of other cells such as macrophages and B cells.

The various cell types of the immune system are commonly characterized by the cell surface cluster determinant (CD) markers they possess. For example, all T cells possess the CD2 marker (Hammerberg and Schurig, 1986). Cluster determinant markers are commonly used to define the lymphocyte population present in a particular tissue or body fluid. The distribution of CD markers can also be utilized to study the development of particular classes, or even subclasses, of lymphocytes as an animal matures.

In swine, CD markers that are commonly utilized to describe lymphocyte populations include, but are not limited to: CD2, expressed by all T-lymphocytes (Hammerberg and Schurig, 1986), CD4, expressed by most T lymphocytes involved in the helper type function (Pescovitz et al., 1984) and CD8, expressed by most T-lymphocytes involved in cytotoxicity (Jonjic and Koszinowski, 1984). In addition, surface bound immunoglobulin, Ig, is commonly used as a marker for B-lymphocytes. Distribution of these lymphocyte subpopulations were evaluated in the thymus, spleen, and peripheral blood of swine from birth to 40 weeks of age by Joling et al. (1994) using CD markers. In the thymus, little change in lymphocyte populations occurred over time, with the majority of thymocytes expressing either CD4, CD8, or both. In the spleen and blood the percentage of CD2 and Ig positive cells increased with time. The majority of the increase in CD2 containing cells was due to an increase in CD8 positive cells (Joling et al., 1994). Unlike other species, swine

possess a significant number of CD4CD8 positive T-lymphocytes in blood and other lymphoid tissues (Joling et al., 1994). These cell types are memory cells. The characterization of the distribution of lymphocytes in a particular tissue is a valuable tool for assessing the developmental and immunological status of swine.

Development of the Organs of the Immune System

The primary and secondary lymphoid organs are tissues where lymphocytes interact with other cells and either undergo further maturation or initiate an immune response. The bone marrow and thymus are the primary lymphoid organs in swine and humans. Both B and T lymphocytes originate in the primary lymphoid organs. The secondary immune organs include the spleen and lymph nodes. It is at these sites that the lymphocytes interact with antigens.

The initiation of hematopoiesis in the yolk sac of the pig has been detected on day 16 of gestation. On day 18 of gestation, approximately 3 million stem cells and 10 million erythroid cells are found in the yolk sac (Tlaskalova-Hogenova et al., 1994). Subsequent development of the fetal immune organs follows a consistent pattern and is governed by cytokines in conjunction with proteins of the extracellular matrix such as fibronectin.

The thymus is the first lymphoid organ to develop and is followed closely by the secondary lymphoid organs, and subsequent appearance of immunoglobulin-containing cells. The thymus develops by day 40 post-conception in the pig (Tizard, 1996).

Progenitor B cells in the fetal pig develop from hematopoietic stem cells under the control of cytokines. Fetal pigs can produce antibodies to parvoviruses and reject allografts

by 58 days (Tizard, 1996) and demonstrate a significant increase in the number of circulation B cells between 70 and 80 days of gestation. In contrast, natural killer cell activity does not develop until several weeks post birth.

The ability of the fetus to respond to antigens develops very rapidly after the lymphoid organs appear but not all antigens are equally capable of stimulating fetal lymphoid tissues. Tlaskalova-Hogenova et al. (1994) observed in cultivated liver and spleen cells from fetuses and precolostral germ free pigs a sequential onset of in vitro responsiveness to different polyclonal B cell activators, including lipopolysaccharide, pokeweed mitogen, suspension of killed bacteria (*Staphylococcus aureus* Cowan and *Escherichia coli*), dextran sulfate and *Nocardia* Delipidated Cell Mitogen (NDCM, a stimulant of B cell proliferation and differentiation). During the first stages of lymphatic system development, NDCM can stimulate IgM and IgG formation (Barot-Ciorbaru et al., 1985). Later in pregnancy a response to *Staphylococcus* and *Escherichia coli* could be detected, but PWM was not capable of stimulating a response until birth. These results would imply that the severity of a dam's infection on the offspring would be highly dependent on the type of organism involved and the state of immunological development of the fetus. An example of such a phenomenon is parvovirus. Fetal pigs receiving parvovirus prior to day 5 of gestation are usually aborted or stillborn. In contrast, after day 72 of gestation, the pig will normally develop high levels of antibodies to the virus and survive (Tizard, 1996).

Development of Phagocytic Capability

In the fetal pig, blood leukocytes collected at 87 to 90 days post conception are capable of phagocytosing particles, but are not fully capable of either virocidal or bactericidal activity. This activity is acquired gradually after birth. Distribution of macrophages also changed after birth. At birth, the neonate possesses few pulmonary macrophages. During the first few days of life, monocytes migrate and adhere to the pulmonary capillary endothelium and differentiate into macrophages. The result of this change in distribution is demonstrated by clearance of most blood particles (75%) in the newborn pig occurring via the liver and spleen. In contrast, by 2 months of age 75% are cleared by the lung (Tizard, 1996).

Transfer of Antibodies from Dam to Offspring

At birth the offspring is immediately faced with an array of microorganisms that it must successfully control or it will not survive. In swine, the immune system is fully developed at birth, but it is not fully functional for several weeks. The immune response mounted by the offspring at this time is typically a primary response with a prolonged lag period and low concentrations of antibodies. During this period of high vulnerability, the offspring relies on maternal antibodies for survival.

The mode of transfer of maternal antibodies to the offspring is partially determined by the placental structure. Since the placenta of the pig is of epitheliochorial structure, transplacental passage of immunoglobulin molecules are totally prevented (Tizard, 1996). Thus, the neonate is entirely dependent on colostral transfer of passive immunity. Failure to

passively transfer maternal immunoglobulins via colostrum to kid goats, a species with similar placental immunoglobulin transfer limitations, leads to increased offspring morbidity and mortality. Serum immunoglobulin G concentration for healthy kids on days 2 to 4 postpartum has been shown to be 49 and 51% higher, respectively, than those requiring treatment or that died (O'Brien and Sherman, 1993).

Porcine colostrum contains approximately 76% immunoglobulin G, 17% immunoglobulin A, and 7% immunoglobulin M (Klobasa and Butler, 1987). Numerous factors affect the concentration of immunoglobulins in sow colostrum including breed, parity, and feed (Inoue et al., 1980 and Inoue 1981). Any factors that would limit the lymphocyte production of immunoglobulins in the sow, such as inadequate amount of DNA necessary for B cell synthesis, would impact the colostral Ig content.

Folic Acid Characteristics and Metabolism

Folic acid is a generic term used to describe a group of naturally occurring pteroylglutamates and related compounds that exhibit similar biological activity in one-carbon metabolism. Folic acid, and its related compounds, were originally investigated due to their anti-anemia actions. Factors M, U, R, and vitamin Bc are a few of compounds investigated in the mid to late 1930's due to their ability to prevent anemia in humans, monkeys, or chickens and were later found to be folic acid related compounds. In 1943 folic acid was isolated from the liver and structurally characterized (Maynard and Loosli, 1969).

Folic acid (pteroylmonoglutamate, PteGlu) consists of a 2-amino-4-hydroxy-pteridine moiety linked via a methylene group at the C-6 position to a p-aminobenzoyl-glutamate

moiety. Folic acid is a yellow-orange, tasteless, and odorless crystalline powder. It is insoluble in alcohol, ether, and other organic solvents, but is highly soluble in hot water. Folic acid is stable to heat in neutral and alkaline solution but unstable in acid and is light sensitive. Large losses of folic acid in feed can occur during processing such as heating and oxidation (Gregory, 1989). Stabilization of folic acid during storage is accomplished by storing in the absence of oxygen or in the presence of reducing agents such as ascorbate.

Most of the folic acid in natural feedstuffs and tissue coenzymes is of the 5,6,7,8-tetrahyrofolic acid species, in which the pteridine ring is reduced, conjugated with varying numbers of glutamic acid molecules, and possibly contains one-carbon substitutes. Polyglutamates found in tissues usually contain less then 7 glutamyl residues (Gregory, 1997). In contrast, synthetic folic acid is in the monoglutamate form.

Dietary polyglutamate must be enzymatically hydrolyzed to the monoglutamate form, via pteroylpolyglutamate hydrolases, also referred to as folic acid conjugases, prior to transport across the intestinal mucosa. Both swine and humans possess conjugase activity in the brush border membrane of the jejunal mucosa (Gregory, 1997). Swine folic acid conjugase exhibits similar properties to that of humans, although quantitatively this enzyme is more active in the human intestine (Wang et al., 1985).

Once enzymatically hydrolyzed, pterolymonoglutamate is sequestered by membrane-associated folate-binding proteins and transported across the brush border membrane via a carrier mediated transport process. At high concentrations (> 5 umol/L), a nonsaturable mechanism involving passive diffusion has been demonstrated to contribute to folic acid absorption (Mason et al., 1990). The optimal pH for transport of pterolymonoglutamate into

the intestine is 5.5-6.5 (Zimmerman et al., 1989).

After absorption, dietary folic acid is transported in plasma as monoglutamate derivatives. In humans the predominate form of folic acid in the blood is 5-methyltetrahydrofolic acid, but dramatic differences exist in the plasma folate distribution in various species. Unlike in humans, two forms of folate are present in the serum of swine: tetrahydrofolate and 5-methyl-tetrahydrofolate, with the primary form being tetrahydrofolate (Natsuhori et al., 1991). Humans, rabbits, dogs, cows, and horses have no tetrafolate observed in plasma, while only trace amounts of tetrahyrofolates are found in plasma from rats and mice (Natsuhori et al., 1991). Unlike 5-methyltetrahydrofolic acid, which is a relatively stable compound, tetrahydrofolate is labile and very susceptible to oxidation and degradation. Two forms of folate binding proteins have been discovered in swine plasma. Ninety-eight percent of the endogenous plasma tetrahydrofolate in swine is bound to a high affinity binding protein, which greatly increases its stability (Sasaki et al., 1996). Alternatively, albumen also serves as a low affinity / high capacity binding protein but does not offer the protection from degradation (Sasaki et al., 1996). The presence of this high affinity binding protein in swine plasma may actually govern the species specificity of plasma folate distribution due to its ability to enable tetrahydrofolate to be stable (Sasaki et al., 1996). Possible hypotheses as to why swine possess this unique distribution of plasma folate include the presence of this high affinity binding protein, increased synthesis or release of tetrahydrofolate from tissues into plasma, and decreased excretion.

Once in the circulation, folic acid is subsequently transported to the liver and peripheral tissues. Within the tissue, the polyglutamate form of folic acid is resynthesized by

the enzyme folic acid polyglutamate synthetase. The active forms of folic acid in the tissues contain either a formyl, methyl, or methylene group attached to the number 5 and/or 10 nitrogens. The primary tissue coenzyme form of folic acid is tetrahydrofolic acid, while the main storage form is 5-methyltetrahydrofolic acid. Folic acid is widely distributed in the tissues of the body, although approximately one-third of the folic acid store in the rat is in the liver (Clifford et al., 1990).

Bioavailability of Folic Acid

According to Gregory (1997) the major factors that affect the bioavailability of folic acid include: 1) dietary form, 2) type of food and food composition, 3) food processing, 4) digestive physiology, and 5) effects of drugs. Since swine are not typically administered drugs that are known to interact with folic acid metabolism, this topic will not be discussed further.

The dietary forms of folate monoglutamates is thought to affect both absorption and post-absorptive retention. Gregory et al. (1992) demonstrated that in folate-saturated humans, significant differences existed among four dietary monoglutamyl folates with respect to post-dose urinary excretion. Matte and Girard (1994) evaluated the bioavailability of folic acid in different grain diets for pigs, using serum pteroylglutamate after ingestion as an indicator of bioavailability. No relationship between the level of dietary pteroylmonoglutamic acid and postprandial concentration of serum pteroylglutamate was found for any dietary source except wheat (Matte and Girard, 1994). This would imply that serum folic acid concentration is not an adequate assessment of bioavailability of dietary folic acid in swine.

Folic Acid Excretion and Catabolism

The amount of intact folic acid secreted by humans and animals is considerably less than ingested, implying the occurrence of catabolism prior to excretion. Cellular catabolism of folate in the rat and human has been demonstrated to occur via cleavage of the C-9-N-10 bond of the molecule producing pterines and *p*-aminobenzoylglutamate (pABGlu) (Murphy et al., 1976 and Murphy et al., 1978). The majority of the resulting pABGlu is subsequently acetylated to *p*-acetamidobenzoylglutamate (apABGlu) (Murphy et al., 1976).

It is assumed that the daily requirement for folic acid is a function of the rate at which catabolism occurs. Therefore, quantification of the rate of catabolism and familiarization with the factors that affect this rate would enable a better estimation of dietary needs. It has been estimated that under normal dietary conditions, humans catabolize only about 100 ug folate per day (McNulty et al., 1987). McNulty et al. (1987) did not observe an increase in rate of catabolism in normal human subjects supplemented with 5 mg folic acid per day. In contrast, Kownacki-Brown et al. (1993) demonstrated that in adult male humans, excretion of both intact folate and the catabolite pABGlu increased after a 7-day supplementation with folic acid, although the increase in folic acid excretion was greater (17 fold increase) compared to the increase in pABGlu (1.7 fold increase).

The rate of catabolism of folic acid increases in high demand situations, such as pregnancy and rapid growth. McNulty et al. (1993) demonstrated an increased rate of folic acid catabolism during pregnancy in the rat in which apABGlu concentration peaked at d 18 of gestation at levels up to three times those of non-pregnant animals. Similar results have been observed during pregnancy in humans (McPartlin et al., 1993). The rate of folic acid

catabolism also increases during rapid periods of growth. McNulty et al. (1995) observed a significant decrease in urinary catabolite excretion in growth restricted rats fed 50% of the required dietary intake for normal growth. Post-dietary restriction, apABGlu concentration and growth rate both increased markedly and even tended to exceed those of normally growing rats of comparable age. Similar results were obtained by Clifford et al. (1993) who demonstrated when folate-depleted rats were repleted with various levels of folate, a two-phase regression model best describes the relationship between dietary folate and growth. In this case, a positive correlation was observed at low dietary levels of folic acid, while little or no correlation was observed at high levels, presumable when the dietary requirement for folic acid had been exceeded (Clifford et al., 1993).

Folic Acid Functions

In its various active forms, folic acid acts in numerous reactions in the body to accept, transfer, and facilitate the enzymatic oxidation or reduction of one carbon units such as: methyl, formyl, and formate groups. Examples of reactions folic acid is involved in include: catabolism of threonine and histidine, methylation of homocyteine to methionine, interconversion of serine and glycine, synthesis of choline, and synthesis of purine and pyrimidines. The role of folic acid in the synthesis of the nucleotide bases will be the primary focus of the remainder of this review.

Due to its fundamental role in DNA and RNA synthesis, folic acid is crucial for rapidly dividing cells such as fetal tissues, placental tissue, and cells of the immune system. Folic acid, in the forms of 5,10-methenyl-tetrahydrofolate and 10-formyltetrahydrofolate, is

necessary for the introduction of carbons -2 and -8, respectively, in the purine ring. Folic acid is also required for the synthesis of the pyrimidine bases via the action of 5,10-methylenetetrahydrofolate in the methylation of deoxyuridylic acid to thymidylic acid, which is further converted to the pyrimidine base thymidine (Herbert and Das, 1976).

Deficiencies of folic acid are associated with inability of the body to perform the critical reactions involving one-carbon metabolism, which includes the impairment of DNA synthesis. Severe folate deficiency is associated with megaloblastic anemia and impaired development of enterocytes in the intestinal mucosa (Klipstein et al., 1973). Folic acid deficiency of rapidly growing rats has also been shown to result in growth depression and anemia. Repletion of these animals results in restoration of growth in direct proportion to the level of supplementation (Clifford et al., 1989). It is hypothesized that deficiency of folic acid during pregnancy would reduce the dam's ability to synthesize the necessary DNA for both fetal and maternal tissues.

Role of Folic Acid During Pregnancy

Due to the rapid rate of DNA synthesis, folic acid is a key nutrient during pregnancy. Administration of folic acid during gestation increased litter size (Lindemann and Kornegay, 1989, Matte et. al., 1984b, Friendship and Wilson, 1991), individual pig weight and length, and total fetal protein and RNA content (Harper et al., 1996) in sows, increased birth weight and maternal weight gain in Squirrel Monkeys (Rasmussen et al., 1980), and increased fetal and placental weight, RNA, DNA and protein content in rats (Morgan and Winick, 1978). In turkeys, addition of folic acid to diets fed to laying hens increased the folic acid content of

the dried egg yolk, egg weight, and poult weight compared to those of hens fed diets unsupplemented with folic acid (Robel, 1993).

However, inconsistencies do exist in the literature as to the effect of folic acid administration on swine reproductive performance. Thaler et al. (1989) observed an increase in litter size and weight at birth and 14 days of age in gilts. Matte et al. (1984b) also observed an increase in litter size in gilts supplemented with folic acid but, neither Matte et al. (1993) nor Easter et al. (1983) observed any effect of supplementing folic acid to gilts or Harper et al. (1994) to sows. These differences may have been due to: differences in dietary folic acid levels; 0.2 (Easter et al., 1983) versus 1.65 and 6.62 mg/kg diet (Thaler et al., 1989), or unusually high pre-gestational folic acid containing diets; .96 mg/kg diet (Matte et al. 1993) versus .3 mg/kg diet recommended by the NRC (1988), potentially creating large folic acid stores in the sow's body.

Although it is hypothesized that the folic acid requirement increases during pregnancy, it is not likely that the increased need is solely due to folic acid content of the fetus, or fetuses, and placenta. The increased estimated need for folic acid during pregnancy may also be due to increased catabolism of folic acid. The rate of folic acid catabolism increases during pregnancy by 300% in rats and peaks on gestation day 18 (McNulty et al., 1993). In humans folic acid catabolism increased by 120% during the second trimester of pregnancy (McPartlin et al., 1993). It is hypothesized that this increased folic acid catabolic rate is associated with a rapid rate of nucleotide synthesis necessary for fetal and maternal hyperplasic growth that occurs during pregnancy. This hypothesis is supported by research in which rats fed .0018 g folic acid/kg diet from 14 days pre-mating through gestation day 21

produced litters with significantly larger fetuses, and fetuses with significantly higher RNA, DNA and protein content than rats fed a folic acid-free diet (Morgan and Winick, 1978). Similar effects were observed in turkeys in which egg and poult weights were significantly increased by dietary folic acid supplementation of the hen (Robel. 1993).

During pregnancy, sow serum folic acid levels decrease from prebreeding levels of 100 ng/ml, to minimal levels of 40 mg/ml on day 60 of pregnancy (Matte et al., 1984a). The decline in plasma folate during pregnancy is primarily due to a decrease in tetrahydrofolate, while little or no change in 5-methryltetrahydrofolate occurs (Natsuhori et at., 1996). The decline in serum folic acid levels can be prevented with injections of 15 mg folic acid during breeding and gestation (Matte et al., 1984, Matte et al., 1994), and it is hypothesized that supplementation with folic acid would allow greater fetal or maternal DNA or RNA accretions to occur in animals with insufficient folic acid intake and body stores.

At birth, the level of total plasma folate is significantly higher in the newborn than the dam in swine. In contrast to the dam, the primary form of folate in the neonate is 5-methyl tetrahydrofolate (Natsuhori et al., 1996). As the pig matures the ratio of 5-methyltetrahydrofolate to tetrahydrofolate declines, as does the total folate content, such that by approximately 24 days of age the primary form in the plasma is tetrahydrofolate (Natsuhori et al., 1996). Possible explanation for the rapid decline in swine plasma folate and the shift in primary form include: an increase in blood volume after birth due to nursing, low levels of maternal milk folate (Matte and Girard, 1989 and O'Connor et al., 1989) and increased tissue absorption of plasma folate.

Assuming that all folic acid catabolic products result from nucleotide synthesis during

gestation, and synthesis of one unit of DNA has the same requirement for folic acid as one unit of RNA, the requirement for maximal hyperplasic growth of a litter of 7 rat fetuses, associated placentas and maternal tissue can be estimated. For this calculation the maximum catabolic rate of folic acid during gestation in the rat, which occurs on gestation day 18 (McNulty et al., 1993), and the estimated fetal DNA and RNA accretion rates during the corresponding time period (Morgan and Winick, 1978) are utilized. An estimation of maternal hyperplasic growth, including all non-fetal tissues, during pregnancy of 3% of maternal weight gain is used based on the increase in placental and uterine weights in swine, and the assumption that 50% of the weight increase in these tissues are due to hyperplasia. Since no increase in mammary gland DNA is observed prior to day 75 of gestation in the gilt (Kensinger et al., 1982), no additional folic acid is required for hyperplasic mammary gland growth. The amount of folic acid required for synthesis of DNA or RNA in the gestational rat is calculated to be 3.81 nmol folic acid per mg of DNA or RNA.

Assuming that the folic acid required per unit of DNA or RNA synthesized in the gravid sow is equivalent to the requirement in the gravid rat, the folic acid needed for the period of rapid hyperplasic growth between days 50 and 63, can also be calculated. It is estimated that for a gilt with a litter of 10 pigs, 1.80 mg of folic acid per day is required for fetal and maternal tissue growth during the period between gestation day 50 and day 63. This would equate with a minimum dietary requirement of .9 mg/kg diet for a sow consuming 2 kg feed/day. Given the variability of estimated bioavailability of folic acid in typical swine feedstuffs (Matte and Girard 1994), a dietary concentration of .9 mg/kg diet may still be significantly below the level necessary to meet the absorbed nutrient needs. Yet, this

estimated dietary concentration is over three times the current NRC recommendation for this time period.

It is not clear how long this rapid rate of hyperplasic growth continues in the sow. The fetal DNA accretion rate during early stages of pregnancy, day 0 through day 30, is only .385 mg DNA/day. This is less than 1% of the estimated accretion rate during the period day 50 through day 63. The total accretion rate post day 63 is not yet known, although it has been shown that the muscle DNA concentration and total liver DNA content continues to increase through day 110 of gestation (Hakkarainen, 1975). As the length of the rapid DNA accretion period increases, the potential for a folic acid deficit also increases.

Role of Folic Acid on the Immune System Development

Prenatal folic acid supplementation also has the potential to increase offspring postnatal performance by increasing immune system development and colostral immunoglobulin content. Development of the fetal immune system occurs at the previously discussed folic acid deficient period between days 40 and 60 of gestation. Depletion of sow folic acid stores, or creation of a folic acid deficiency in the neonate during this critical time of gestation, may hinder the pigs ability to mount an immune response postnatally.

Fetal malnutrition in humans can cause abnormalities in: cell-mediated immune response (decrease in the number of T-lymphocytes, binding affinity, and ability to synthesize DNA in the presence of mitogen), complement system, phagocytes, mucosal secretory antibody response, antibody affinity, and impaired delayed hypersensitivity (Chandra, 1975). The most consistent abnormalities in humans are seen in cell-mediated

immunity of the offspring, which may be impaired for a period of months to even years after birth (Chandra, 1992).

The effects of folic acid deficiency on both cell-mediated and humoral immunity have been investigated in various non-swine species. Gross et al. (1975) examined the affect of a folic acid deficiency, similar to the type of deficiency commonly seen with pregnancy, on cell-mediated immunity in women. Women experiencing folic acid deficiencies were differentiated by megaloblastic changes in bone marrow specimens, the presence of megaloblastic anemia symptoms in peripheral blood smears (presence of macrocytic red cells and multilobed polymorphs), and serum folic acid levels below 6.0 ng/ml in the presence of normal serum B_{12} levels. The range of normal serum folate in this laboratory was 6 to 21 ng/ml. The primary effects of the folic acid deficiency on cell-mediated immunity included decreased blastogenic response of T-lymphocytes and a negative dinitrochlorobenzene (DNCB) skin test (a measurement of the responsiveness of the cell-mediated immune system). Similar results were observed in rats fed either a control or folic acid-free diet from weaning. After three months on the folic acid free diet rats had significantly lower serum folic acid levels (24 ng/ml folic acid-free versus 100 ng/ml control), decreased body weight, decreased spleenic lymphocyte cytotoxic activity (measured in-vitro), and decreased sensitivity and number of T-cells (Williams et al., 1975). Gestational folic acid deficiency can also affect humoral immunity, as demonstrated by a decreased antibody response to some antigens, although it is not clear if this response is due to decreased number of memory cells produced after an antigenic stimulation and/or a decrease in immunoglobulin production per cell (Dhur et al., 1991).

Role of Folic Acid in Postnatal Performance

Postnatal needs for folic acid in the sow include transfer of folic acid to the neonate via nursing and production of colostral immunoglobulins. Sow milk folic acid levels are low compared to many other species (O'Connor et al., 1989). Moreover, sow milk contains approximately the same concentration of folic acid as that of plasma. This is not typical of other species that maintain plasma folic acid levels significantly below milk levels (O'Connor et al., 1989). This may be interpreted as a sign of a folic acid deficiency in the sow.

As lactation progresses, sow serum folic acid levels increase from approximately 30 ng/ml at farrowing to approximately 55 ng/ml after 28 days of lactation (Matte and Girard., 1989). This is possibly due to the increased feed intake during lactation and/or the decline in plasma volume. In contrast, as lactation progresses milk folic acid levels decline by approximately 10%. This decline is reflected in the plasma folic acid content of the pigs which increased from days 2 to 16 of age and then gradually decreased (Matte and Girard., 1989).

Folic acid is also important to assure adequate production of colostral immunoglobulins. Since the pig can not absorb immunoglobulin in utero, optimizing colostral immunoglobulin content and absorption by the neonate is essential. The predominant immunoglobulin in sow colostrum is IgG, which accounts for approximately 80% of the total. Both IgA and IgM are also present in significant quantities in sow milk. Synthesis of the DNA and RNA necessary to produce the B-cells required to synthesize these immunoglobulins requires folic acid. Since lymphocytes rapidly turn over in the body, this particular demand for folic acid has the potential to be great. Deficiencies in folic acid at the

time of immunoglobulin synthesis and secretion could detrimentally affect the pig's ability to withstand immune challenges.

Benefits of Folic Acid Supplementation

Given the estimated total body folic acid store of 12.19 mg of folic acid in a gilt at breeding, the folic acid requirement of 3.62 nmol/mg of DNA or RNA accretion in a pregnancy producing 10 offspring, and the estimated average fetal DNA accretion rates of: .385 mg/d during d 0-d 30, 3.05 mg/d during d 31-d 50 and 47.2 mg/d during d 50-d 63, it would require only 52 days for the folic acid stores of a gilt to be completely depleted by the needs for hyperplasic growth. This calculation is made assuming that dietary folic acid, at NRC recommended .3 mg folic acid per kg diet, is used only to meet the gilt non-gestational folic acid requirement, such as interconversion of serine and glycine, histidine degradation, and synthesis of methyl groups for compounds such as methionine, choline and thymidine. Any additional requirement such as an immune challenge or the need to deposit folic acid into fetal tissue or colostrum would increase the depletion of body stores. In order to meet the gestational requirement for optimal hyperplasic fetal and maternal growth through gestation day 63, the gilts would need to eliminate over 2.5 fetuses from a litter of 10 or reduce development of each fetus below the maximal level.

In a positive perspective, by supplementing folic acid to a level required by the gilt for maximum hyperplastic growth, the producer would have the potential to increase the number of pigs born alive or the muscle cell number at birth by 20%. The economic value of increasing litter size would be $1.74 per additional pig at birth (given 2.5 litter/year, feed cost

$.065/lb. litter size of 10. and fixed cost of $.15/day). If litter size was maintained but rather total muscle cell fibers were increased. the additional value would be $7.28 per pig at market for each 10% change in muscle content (given 1% additional muscle = -1 day to market, -18 lbs feed/day and additional $0.40 per pig carcass value). Additional economic returns could be realized by increasing the immune capacity of the offspring. Each percentage unit reduction in pig mortality resulting from enhanced immune capability of the offspring would result in an additional pig marketed per litter of 10 farrowed. This does not include additional economic benefits from reduced morbidity of the offspring.

Given the high economic demands placed on today's commercial hog producers, an increase of such magnitude would greatly increase the producer's potential viability in the industry at a minimal cost.

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CHAPTER 2. THE EFFECTS OF GESTATIONAL FOLIC ACID SUPPLEMENTATION OF SOWS ON OFFSPRING IMMUNE ORGAN DEVELOPMENT AND POSTNATAL IMMUNE RESPONSE

A paper to be submitted to Journal of Animal Science
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Abstract

Primiparous sows (113±27 kg BW) from nineteen pairs of littermates were penned individually and fed daily 1.9 kg of a basal, low folic acid diet (.28 mg/kg) supplemented with 0 or 8 mg folic acid from coitus to parturition. All sows were fed the basal diet for 112 days prior to mating to minimize body folic acid (FA) stores. Sows were synchronized and artificially inseminated within sow litter pair to the same boar. At 5±2 d post-insemination, ovulation rate was determined via laparotomy. At birth, two pigs in each litter were sacrificed. Litters were standardized to 10±2 pigs and sows were self-fed a nutritionally adequate diet. At 11±2 d of age, two pig per litter was weaned, penned individually, self-fed a nutritionally adequate diet, and blood samples were collected for immunological assessment during a 56 d period. Pigs were then administered 1 mL of 20% sheep red blood cell (SRBC) i.m and agglutination titers determined weekly for 49 d. Sow serum FA concentration, an indicator of body FA stores, were determined weekly and declined from 69 to 51 ng/mL during the 112 d depletion period. During pregnancy, serum FA concentration further declined thru mid pregnancy (d 70) then increased to d 84 or 91, finally declining as parturition approached. Gestational FA supplementation elevated ($P<.01$) sow serum FA concentration. Gestational FA supplementation did not alter immunoglobulin (IgA, IgG)

concentrations in sow serum on d105 of pregnancy or in colostral whey or pig serum at parturition. Gestational FA supplementation did not affect fetal survival (number of pigs born alive divided by the number of corpora lutea, multiplied by 100), pigs born/litter (8.10 vs. 8.15), litter birth weight (10.49 vs. 11.52 kg), nor pig thymus and spleen weight, or DNA and protein content at birth. The percentage of CD2 positive lymphocytes in peripheral blood of pigs from FA supplemented dams was greater on d 0 but not d 21 postweaning resulting in a FA supplementation by day interaction. Gestational FA supplementation also increased the offspring lymphocyte blastogenesis rate upon stimulation with PHA at d 0 but not d 28 or 56 postweaning, resulting in a FA by day interaction ($P \leq .05$). FA supplementation of the dam resulted in greater ($P < .05$) serum agglutination titers in pigs following a secondary SRBC challenge. Based on these data, FA status of the gravid dam influences postnatal immune response of the offspring.

Key words: Pigs, Folic Acid, and Immune Response

Introduction

Economically important goals in pork production include the production of healthy, viable offspring that grow muscle rapidly and produce highly muscled carcasses. Numerous environmental factors, including the dam's nutritional status, influence whether the offspring will survive and what proportion of the offspring's genetic capacity for growth will be expressed. It has been demonstrated that postnatal growth in pigs (Dwyer et al., 1994) and immune capacity in humans (Chandra, 1992) can be affected by prenatal growth.

The capability of the immune system to respond to an immune challenge is largely

determined by the number, type, and functionality of immune cells synthesized in the fetus. All cellular elements of the immune system are ultimately derived from the same hematopoietic stem cells, located in the bone marrow of the mammalian fetus (Janeway and Travers, 1996). Further differentiation, proliferation, and maturation of these cells occur in the primary and secondary immune organs. An adequate supply of DNA precursors are necessary for the growth of these hematopoietic cells.

Transplacental passage of nucleotides, nucleosides, and bases is possible in rats (Hayashi and Garvey, 1968). But since the placental structure of the pig is less permeable than that of the rat, mouse, and human, it is hypothesized that placental transfer of DNA precursors is minimal, and therefore these substances must be synthesized in the swine fetus.

Folic acid (FA) has a fundamental role in *de novo* synthesis of DNA (Herbert and Das, 1976). Therefore, the amount of FA available to the dam may influence the amount of hyperplastic growth that occurs in fetal and maternal tissues. Serum FA concentration in sows decline during pregnancy to minimal levels during mid-gestation (Matte et al., 1984), which corresponds with peak periods of hyperplastic growth of immune cells in the fetus (Tizard, 1996). The rate of FA catabolism also has been shown to increase during pregnancy in the rat (McNulty et al., 1993). These findings support the hypothesis that the FA requirement of the sow increases during pregnancy.

The objective of this experiment was to determine the influence of gestational FA supplementation of sows on the immune capacity and subsequent performance of the offspring.

Materials and Methods

Treatments

Primiparous sows were fed daily 1.9 kg of a basal, low folic acid diet (.28 mg folic acid/kg, Table 1) supplemented with 0 or 8 mg folic acid from coitus through parturition. This dietary regimen provided a daily intake of metabolizable energy, amino acids, minerals, and vitamins (except FA) that met or exceeded the estimated nutrient needs of the gravid sow (NRC, 1988). To minimize body FA stores, all sows were fed 1.9 kg/d of the basal diet for 112 d prior to breeding (Tables 1 and 2).

Sow management and procedures

Nineteen littermate pairs of crossbred primiparous sows of Landrace, Yorkshire, Duroc, and Hampshire ancestry were evaluated. Beginning on d 98 of the depletion period, sows within each littermate pair were synchronized by feeding daily 14 mg altrenogest (Regu-Mate®, Roussel UCLAF, Agro-veterinary Division) per sow for 14 days. Following altrenogest withdrawal, sow estrous activity was checked with a boar twice daily (0800 and 1400 h). Littermate pairs were artificially inseminated with semen (Pipestone Artificial Breeders, Pipestone, MN) from the same boar during the initial two or three post estrus detection periods. On the day following insemination, sows within each littermate group were randomly allotted to the dietary regimens of 0 or 8 mg of supplemental FA per day throughout pregnancy.

At 5±2 days post-insemination, sow ovulation rate was determined via laparotomies

Table 1. Composition of basal diet.

Ingredient	% of Diet
Corn, ground	91.58
Casein	3.49
L-Lysine-HCl, 98.5%	0.12
L-Threonine	0.04
Tryptophan/lysine blend[a]	0.266
Dicalcium phosphate	2.79
Limestone	0.10
Salt	0.50
Potassium sulfate	0.50
Trace mineral mix[b]	0.11
Choline chloride, 60%	0.30
Vitamin mix[c]	0.10
Chloratetracycline mix[d]	0.10

[a]ADM tryptosine™ 15/70 contained 55.3% L-lysine, 15% L- tryptophan, 1.75% methionine, 0.5% valine, and 0.15% threonine.

[b]Provided per kg of diet: 10.5 mg Cu, 105 mg Fe, 36 mg Mn, 90 mg Zn, 0.12 mg I, and .30 mg Se.

[c]Provided per kg of diet: 0.75 mg biotin, 30 mg niacin, 44 mg pantothenic acid, 14 mg riboflavin, 1.0 mg pyridoxine, 1.0 mg thiamine, 70 IU vitamin E, 16,000 IU vitamin A, 800 ICU vitamin D_3, 2 mg vitamin K, and 60 ug vitamin B_{12}.

[d]Provides 110 ppm chloratetracycline per kg of diet.

Table 2. Dietary regimens.

Recipient	Stage of Development	Days	Diet	Analyzed FA, mg/kg diet[a]	Feed offered
Sow	Prebreeding	112	Basal (-FA)	.28	1.9 kg/d
Sow	Pregnancy	114	Basal (-FA)	.28	1.9 kg/d
			Basal (+FA)	4.36	1.9 kg/d
Sow	Lactation	11	Corn/SBM	1.18	Ad lib
Pig	Postwean	56	Corn/SBM/Whey	2.36	Ad lib
Pig	SRBC Challenge	42	Corn/SBM	2.07	Ad lib

[a]Folic acid (FA) analyzed via Quantaphase II B12/Folate Radioassay, Bio-Rad.

and the visual counting of the number of corpora lutea present on each ovary. This procedure was performed under a surgical plane of anesthesia obtained by injecting 1 mL per 45.45 kg of a mixture of Telazol (100 mg/mL), Ketamine (50 mg/mL), and Xylazine (50 mg/mL) into the neck region, followed by 3% inhaled halothane. Once the necessary plane of anesthesia was obtained, the level of halothane was maintained at 2-3%. An incision through the skin and underlying tissues of approximately 7.62 cm was made and each ovary was exposed in order to visually count the corpora lutea present. Once skin closure was begun, the level of halothane was reduced to 0% to aid in recovery.

During the depletion and gestation periods, sows were individually penned in .61 x 2.13 m stalls on slotted floors in temperature controlled and mechanically ventilated rooms. Sows were allowed to consume water ad libitum throughout the experiment. Sows were vaccinated for the following pathogens: pseudorabies virus (PR-VAC, Nobel Laboratories, Sioux Center, IA) at 8 and 4 weeks prebreeding, and *Mycoplasma*

hyopneumoniae (RespifendTM. Solvay. Mendota Heights. MN). *Bordetella bronchiseptica* (Atrobac 3. NOBL. Sioux Center. IA). porcine rotavirus. transmissible gastroenteritis virus, *Clostridium perfringens* type C. *Escherichia coli* (Scourshield D. SmithKline Beecham, West Chester. PA) and swine influenza virus (MaxiVac-FLU. SyntroVet. Lenexa. KS) at 4 and 2 weeks pre-parturition.

Serological titers for the following pathogens were analyzed for in 16 representative sows at 8 weeks prebreeding and 6 weeks prefarrowing: *Actinobacillus pleuropneumoniae*, *Mycoplasma hyopneumoniae*. porcine reproductive and respiratory syndrome virus, pseudorabies virus. swine influenza virus. and transmissible gastroenteritis virus as described by Williams et al. (1997).

On day 109±4 post-breeding. sows were moved to sanitized farrowing rooms and penned individually in 1.52 x 2.13 m farrowing stalls on slotted floors. Sows were maintained on the previously assigned dietary regimen until parturition occurred. In an effort to minimize the time period required for all experimental animals to be farrowed. therefore creating a more consistent level of environmental antigen exposure for the neonatal pigs. all of the synchronized bred sows that had not farrowed by the third day of the farrowing period were injected i.m. with 2 mL of dinoprost tromethamine (Lutalyse®, 5 mg dinoprost/mL) to stimulate farrowing. Post-farrowing. all sows were fed a fortified corn-soybean meal diet supplemented with minerals and vitamins (including FA) at levels that met or exceeded the estimated needs of high producing lactating sows (Table 2). Lactating sows were allowed to consume feed and water ad libitum.

Folic acid status monitoring

Sow serum FA concentrations were determined weekly in 15 representative sows during the 112 d depletion period (with the exception of depletion d 105), and weekly in all sows on d 28 to 105 of pregnancy, via radioimmunoassay (Bio-Rad Quantaphase B12-Folic Acid kit, Hercules, CA). Serum FA content also was determined in one pig from each litter at birth (presuckling). The FA radioimmunoassay was able to recover 91.5% of the FA in a spiked serum sample and to detect a minimal concentration of 0.1 ng of FA/ml (Bio-Rad, Hercules, CA). The inter- and intra-assay coefficients of variation of the FA radioimmunoassay were 5.47 and 10%, respectively.

Sow immunological assessment

Immunoglobulin A (IgA) and G (IgG) concentrations in sow serum on d 105 of pregnancy and colostral whey at parturition were analyzed via Ig-ELISA assay (Rajaraman et al., 1997) using IgA and IgG antibodies provided by Dr. Butler, University of Iowa. Colostral whey was isolated via centrifugation (50,000 G for 60 min at 4^{0}C) from hand expressed colostrum samples collected from the second and fourth functional gland prior to the initial nursing bout.

Offspring management procedures

Each live pig farrowed was caught prior to nursing the dam, sexed, weighed, numbered via ear notches, and the umbilical cord tied. All pigs born dead also were weighed. At birth, two pigs from each litter were selected based on the proximity of their

weight to the mean weight of the previous pigs farrowed and sacrificed prior to colostrum consumption, via a jugular injection followed by intracardial injection of sodium pentobarbital. These pigs were exsanguinated and frozen for subsequent immune organ dissection. The remaining pigs in each litter were allowed to nurse following weighing. Litters were standardized to 10±2 pigs by 1 d post farrowing in an attempt to minimize variability in milk demand among litters. Whenever possible, pigs were transferred from a sow on the same gestational treatment. If this was not possible, pigs were brought in from a neutral sow, that had not received the dietary treatments during pregnancy. All pigs were injected i.m. with 5 mg/kg body weight of ceftiofur sodium (Naxcel®, Upjohn, Kalamazoo, MI), on d 1, 3, 5, 7, 9, and 11 of lactation to standardize the health status of the pigs at weaning.

Additional litter processing that occurred within 24 hours of birth included clipping of needle teeth, cutting of tails, and administration of 100 mg iron dextran. At 7±3 d post farrowing, all male pigs were castrated. Pigs were not allowed access to supplemental feed or water during the lactation period, but did have access to thermostatically controlled heating pads as a source of supplemental heat.

At 11±2 d of age, two additional pigs (native to the birth sow) per litter were selected based on the proximity of their weight to the mean weight of the litter. These pigs were weaned and penned individually on slotted floors in .48 x 1.22 m pens in rooms located within the original sow complex for 56 d postweaning. In order to stimulate the immune system with a general immune challenge, the rooms were not cleaned (power washed) or disinfected prior to the occupation.

Pigs were allowed to consume ad lib a commercial milk based diet (Soweena® Litter Bites #1, Merrick's, Middleton, WI) for 7 d and then a fortified corn-soybean meal-whey diet. These diets were formulated to meet or exceed the estimated nutrient needs of 5 to 10 kg pigs (NRC, 1988, Table 2). Body weights and feed consumption were determined weekly for 56 days.

At 85±4 d of age, one pig from twelve litters per sow treatment was moved into a separate, mechanically ventilated room within the original sow complex where they were individually penned on slotted floors in .61 x 2.13 m pens. Pigs were allowed to consume ad libitum a corn-soybean meal diet formulated to meet or exceed the estimated nutrient needs of a 25 to 50 kg pig (NRC, 1988, Table 2). Pig weights and feed intakes were determined weekly for 42 days.

Offspring immunological assessment

Serum IgA and IgG concentrations were determined at birth (before suckling) in one pig per litter (Rajaraman et al., 1997). From the two pigs per litter killed at birth, the thymus and spleen were isolated, weighed, and refrozen for subsequent DNA (Labarca and Paigen, 1980) and protein (AOAC, 1984) content analysis.

Peripheral lymphocyte characterization and blastogenesis rates were utilized to evaluate the effect of gestational FA supplementation on the offspring's immune response to a general immune challenge during the initial postweaning period. Peripheral lymphocyte characterization was conducted on only one pig per litter on d 0, 7, 14, and 21 post weaning via antibody binding and flow cytometry detection of cell surface cluster determinant (CD)

markers (Chou et al., 1996). The antibodies used in this assay and the appropriate dilution of each are described in appendix 1. Samples were analyzed at the Cell and Hybridoma Facility at Iowa State University.

Serum mitogen-induced blastogenesis rates were determined on one pig per litter on d 0, 28, and 56 postweaning as described by Nonnecke et al., (1991) with the following modifications: after centrifugation, the mononuclear leukocyte-enriched buffy coat layer was diluted in 2.5 ml of Hank's balanced salts solution and the working density of the cell suspension was $2.0x10^6$ cell/ml in RPMI-1640 medium supplemented with 2 mM L-glutamine (Sigma Chemical Co, St. Louis, MO), 100 IU/mL of penicillin G sodium, and 100 ug/mL of amphotericin B (Gibco Laboratories). Culture plates were seeded with $1.0x10^6$ cell/mL in a final culture volume of 200 uL and supplemented with fetal bovine serum (heat inactivated, 5% vol/vol, Hyclone Laboratories Inc., Logan, UT). Individual cultures contained either no mitogen (nonstimulated, control cultures) or 10 ug/ml of: concavalin A (ConA, Sigma Chemical Co.), phytohemagglutinin-P (PHA, Sigma Chemical Co.) or pokeweed mitogen (PWM, Sigma Chemical Co). ConA and PHA are stimulators of T-lymphocytes, while PWM is a stimulator of both T and B-lymphocytes. Individual cultures were incubated 66 h at 37^0 C and pulsed during the last 18 h with 18.5 Kbq of methyl-[3H] thymidine. Peripheral lymphocyte characterization and lymphocyte blastogenesis rates were determined on separate pigs from each litter unless inadequate number of pigs was available.

In an effort to determine the offspring's response to a specific antigen challenge, one pig from each of 12 litters per sow treatment were administered 1 mL of a 20% sheep red blood cell solution (SRBC, a T-dependent B cell stimulant) i.m. at 85 days of age (d 0) and

21 days later. These pigs were chosen based on availability of offspring from sow littermate pairs and proximity of the individual pig's performance to the mean performance during the previous postweaning period. Blood samples were collected weekly for 42 days and analyzed for agglutination response to SRBC (Wegmann and Smithies, 1966).

The animal care procedures employed in this study were approved by the Iowa State University Committee on Animal Care.

Statistical analysis

The study was analyzed as a randomized complete block design with sow considered the experimental unit and sow or boar littermate-pair the block. Data collected from multiple offspring from the same litter were averaged for statistical analysis. Data were analyzed by analysis of variance techniques using general linear model (GLM) procedure of SAS (1996). The relationships between particular variables (ie. sow serum FA concentration and immune organ characteristics) were analyzed using linear regression procedures of SAS (1996). The error term used to test the effect of gestational FA supplementation was the FA by block interaction in all analyses except that of the lymphocyte blastogenesis data. Since block x FA interaction and the block x time interactions were not significant in the blastogenesis data analysis, they were dropped from the model for this analysis and the error term used to test the FA and time main effects was experimental error.

Peripheral lymphocyte characterization and blastogenesis rate over time (days) were analyzed as repeated measures. Nonstimulated lymphocyte blastogenesis rates were subtracted from each stimulated blastogenesis rate prior to analysis. Initial primary and initial

secondary agglutination responses were used as a covariate for the primary and secondary SRBC response data and type I sums of squares were used for this analysis. Least square means are reported unless stated otherwise.

Results and Discussion

Feeding the basal low FA diet during the 112 d prebreeding period resulted in a decline ($P<.05$) in sow serum FA concentration from 69 to 51 ng/ml (Figure 1). Premating serum folate values of approximately 52 and 57 ng/ml have been reported in sows by Matte et al. (1996). Based on these data, the goal of minimizing body FA prior to the initiation of the experiment was achieved. Low gestational serum concentrations of folic acid have been associated with low birth weight, increased incidence of fetal–growth retardation (Tamura et al., 1997), and increased pre-term delivery (Scholl et al., 1996) in humans. Folic acid deficiency also has been associated with a reduced cell-mediated immunity in humans (Gross et al. 1975; Dhur et al., 1991).

In the current study, dietary FA supplementation (8 mg/sow/d) during pregnancy increased ($P<.05$) sow serum FA concentration (Figure 1). As pregnancy progressed, serum FA concentration declined to minimal levels at d 70 in both the basal and supplemented groups (21.5 and 47.5 ng/ml, respectively). The serum FA concentrations then increased thru d 84 or 91 and then again declined as sows approached parturition. Matte et al. (1984) observed minimal folic acid concentration of 40 ng/ml on day 60 of pregnancy in sows fed a total of 1.2 mg FA/d. Reductions in blood FA levels have been reported during mid pregnancy in the sow (Matte et al., 1984) and lactating dairy cows (Girard et al., 1989).

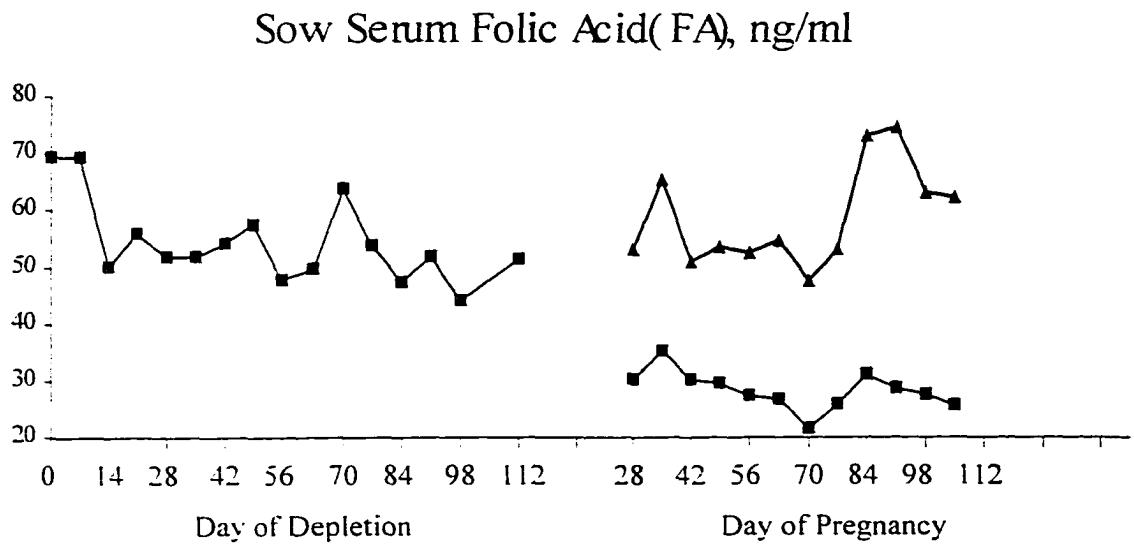

Figure 1. Effect of dietary gestational folic acid (FA) supplementation of 0 (■) or 8 (▲) mg FA/kg diet on serum FA concentration in sows during a 112 d depletion period and 77 d phase of pregnancy. Least square means represent the values of 15 and 39 sows during the depletion and pregnancy stages, respectively. Sow serum FA concentration during pregnancy was influenced ($P<.01$) by gestational FA supplementation.

Gestational FA supplementation of sows also increased serum FA concentrations in pigs at birth (44.2 versus 21.4 ng/ml, Table 3). Serum FA concentrations in pigs at birth were lower than that of sows on d 105 of pregnancy (Table 3). These results are contrary to those of Natsuhori et al., (1996) who observed much higher levels of total plasma folates in newborn pigs compared with nongestating sows fed 0.9 mg folate/kg diet.

The number of corpora lutea determined post coitus (16.0±3.1), fetal survival (number of pigs born alive divided by the number of corpora lutea, multiplied by 100) (44.7±22.4), number of pigs born (8.10 vs 8.15), and litter birth weight (10.49 vs 11.52 kg)

Table 3. Effect of dietary gestational folic acid (FA) supplementation of 0 or 8 mg FA/d on concentrations of immunoglobulin A (IgA) and G (IgG) in sow serum, colostral whey, and pig serum and concentration of FA in sow and pig serum[a].

Criteria		Sow FA supplementation, mg/kg 0	8	SEM
Number of sows		19	20	
Number of pig litters		17	16	
Immunoglobulin Concentrations (mg/ml)				
Sow serum (d 105)	IgA	22.3	19.9	0.9
	IgG	23.8	22.9	1.4
Colostral whey (presuckle)	IgA	14.3	15.1	0.9
	IgG	31.6	27.6	1.1
Pig serum (presuckle)	IgA	0.5	0.6	0.04
	IgG	1.7	2.9	0.31
Folic acid concentration (ng/ml)				
Sow serum (d 105)[b]		25.6	62.2	1.61
Pig serum (presuckle)[b]		21.4	44.2	2.95

[a]Least Square means reported.
[b]Differences due to folic acid supplementation, P<.005.

were not altered by gestational FA supplementation. These results are contrary to those of Thaler et al. (1989) and Matte et al. (1984) who observed increases in litter size due to folic acid supplementation, but in agreement with results of Matte et al. (1993), Easter et al. (1983), and Harper et al. (1994) who did not observed any effect of supplementing folic acid on litter size. The proportion of ova that were fertilized, survived, and subsequently farrowed in this experiment were less than anticipated. Factors that likely contributed to the reduced litter size include: laparotomies performed on d 5 post coitus and the seroconversion of some sows for pseudorabies during the 112 d depletion period of this study. Unfortunately, this reduction in number of fetuses presumable reduced the folic acid demand during pregnancy.

Immediately post-birth, the pig is highly vulnerable to immunological challenges due to a lack of in-utero transmission of maternal immunoglobulins (Tizard, 1996). During this period, the offspring rely on colostral immunoglobulins for survival. Presumably any factors that would limit lymphocyte production of immunoglobulins in the sow, such as inadequate supply of DNA necessary for B cell proliferation, would impact colostral immunoglobulin content.

Gestational FA supplementation did not alter ($P>.05$) concentrations of IgG or IgA in sow serum (d 105 of pregnancy), colostral whey, nor pig serum (presuckling) (Table 3). Significant relationships did exist ($P<.05$) between the IgG and IgA concentration in sow serum and colostral whey ($R^2=.38$ and .52, respectively), and between IgA concentrations in sow and pig serum ($R^2=.19$). No relationship existed, nor was one expected, between immunoglobulin concentrations in colostral whey and pig serum, as the pigs were not allowed to nurse colostrum prior to blood sampling.

Although development of the primary immune organs occurs during early to mid pregnancy in the pig (Tizard, 1996), the time period associated with a decrease in sow serum folic acid levels, no differences (P>.10) were detected in thymus and spleen weight, DNA, or protein content of pigs at birth (Table 4). Neither sow gestational serum FA concentration from d 28 to 105 of pregnancy, nor pig birth serum FA concentration were related to pig thymus and spleen DNA concentrations at birth. Based on these data, gestational FA supplementation did not affect hyperplastic growth of these immune organs in utero. These results are not in agreement with those obtained by James et al. (1992) who observed a reduction in both purine and pyrimidine nucleotide pools in spleen cell extracts from rats fed FA deficient, nucleotide-free diets.

The various cell types of the immune system are commonly characterized by the cell surface cluster determinant (CD) markers they posses. In swine, CD markers that are commonly utilized to describe lymphocyte populations include: CD2, expressed by all

Table 4. Effect of gestational folic acid (FA) supplementation of 0 or 8 mg FA/sow/d on the offspring thymus and spleen traits at birth (presuckle)[a].

Item	Criteria	Sow FA supplementation, mg/d		SEM
		0	8	
No. of litters		17	19	
Spleen	Weight, g	1.39	1.45	.05
	DNA, mg	16.73	16.27	.78
	Protein, g	0.23	0.24	.01
Thymus	Weight, g	2.51	2.71	.13
	DNA, mg	31.75	31.88	2.08
	Protein, mg	0.37	0.41	.02

[a]Least square means reported.

T-lymphocytes (Hammerberg and Schurig, 1986); CD4, expressed by most T lymphocytes involved in the helper type function (Pescovitz et al., 1984); and CD8, expressed by most T-lymphocytes involved in cytotoxicity (Jonjic and Koszinowski, 1984). In addition, surface bound IgM is commonly used as a marker for B-lymphocytes. Unlike other species, swine possess a significant number of T-lymphocytes containing both CD4 and CD8 markers (CD4CD8) in blood and lymphoid tissues (Joling et al., 1994 and Pescovitz et al., 1984).

The percentage of CD2 positive lymphocytes in peripheral blood of pigs from FA supplemented dams was greater on d 0 but not d 21 postweaning, resulting in a FA supplementation by day interaction (Table 5). Since the CD2 marker is present on all T cells, this effect would imply that pigs from sows supplemented with FA initially had a higher percentage of circulating T cells. The percentage of IgM-positive lymphocytes in pigs from FA supplemented dams was less on d 0 but not d 21 resulting in a tendency for a FA supplementation by day interaction ($P<.10$) (Table 5). The reduction in percent of IgM-positive cells observed in offspring from dams on either dietary regimen is possibly due to the maturation of the B cells from IgM expressing cells to IgG expressing cells.

Gestational FA supplementation did not alter ($P>.10$) the percent of peripheral blood lymphocytes expressing the CD4 or CD4CD8 (dual positive) markers in pigs during the 21 d postweaning period but marker expression was affected by number of days postweaning (Table 5). The percent of CD4 positive lymphocytes decreased ($P<.01$) over the 21 d postweaning period while the percent of CD4CD8 positive lymphocytes increased. This would be expected as CD4CD8 dual positive lymphocytes are memory cells that develop as the animal is exposed to antigens. The percent of CD8 positive lymphocytes increased from

Table 5. Effect of gestational folic acid (FA) supplementation of 0 or 8 mg/sow/d on the percent of peripheral lymphocytes presenting the CD2, CD4, CD8, CD4/CD8 and IgM cell surface markers[a].

Lymphocyte Marker	N	Days Post Weaning	Sow FA supplementation, mg/d		SEM
			0	8	
CD2[b]	35	0	47.09	51.53	0.77
	34	7	44.19	34.94	
	34	14	52.33	42.38	
	34	21	51.14	45.78	
CD4[c]	35	0	22.58	25.59	0.38
	32	7	16.94	16.99	
	34	14	15.58	16.32	
	34	21	14.93	15.97	
CD8[c,d]	35	0	6.78	6.14	0.39
	32	7	10.18	8.46	
	34	14	18.75	12.87	
	34	21	12.92	11.45	
CD4CD8[e]	35	0	0.92	0.48	0.25
	32	7	0.60	0.42	
	34	14	1.89	1.18	
	34	21	3.01	3.17	
IgM[e]	35	0	21.19	17.65	0.63
	30	7	12.18	10.37	
	27	14	15.05	12.52	
	31	21	7.68	11.69	

[a]Response over time analyzed as repeated measure. Raw means reported.
[b]FA*Day interaction effect. P<.05.
[c]Days post weaning effect. P<.01.
[d]FA effect. P =.11.
[e]FA*Day interaction effect. P<.10.

weaning thru d 14, then declined by d 21 ($P<.01$). The magnitude of change in CD8 positive lymphocytes tended to be affected by gestational FA supplementation ($P=.11$). These data are in support of the general understanding that the pig is born with a significant population of helper T-cells but do not develop a large population of cytotoxic or memory T-cells until it is presented with viral antigens.

Gestational FA supplementation did not affect ($P>.10$) peripheral blood lymphocyte blastogenesis rate upon exposure to ConA, a T-cell mitogen, or PWM, a B and T cell mitogen (Table 6). Gestational FA supplementation resulted in an increase in offspring lymphocyte blastogenesis rate upon stimulation with PHA, a T cell stimulant, at weaning (d 0) but not at 28 and 56 d postweaning, resulting in a FA by day interaction ($P\leq.05$). Lymphocyte blastogenesis rates induced by each of the three mitogens were highest at weaning and then were reduced significantly at d 28 and 56 post weaning ($P<.05$). Sow gestational FA supplementation did not affect the offspring daily weight gain, feed intake, or gain:feed ratio during the 56 d postweaning growth period (Table 7).

At weaning, pigs are commonly vaccinated against the antigens prevalent to the herd of origin. Assuming that this is the offspring's primary exposure to that particular antigen, any factor that would increase the offspring's secondary response, a response to the live organism originating in the environment, would presumable increase the survival and performance of the animal. Gestational FA supplementation of the dam did not affect the offspring's primary (d 0 to 21) antibody response to the SRBC challenge (Figure 2). But, gestational FA supplementation did increase ($P<.05$) the offspring's secondary (d 21 to 42) antibody response to a SRBC challenge (Figure 2). These results would indicate that the

Table 6. Effect of gestational folic acid (FA) supplementation of 0 or 8 mg/sow/d on offspring mitogen[a] stimulated lymphocyte blastogenesis rate[b] postweaning.

Criteria	Days Post Weaning	Sow FA supplementation, mg/d		SEM
		0	8	
No. litters	0	13	15	
	28	16	15	
	56	16	15	
Lymphocyte blastogenesis rate (counts per minute, cpm)				
ConA[c]	0	70,522	78,903	1,620
	28	37,408	34,209	
	56	41,153	37,621	
PHA[d]	0	46,005	63,491	1,966
	28	42,863	40,058	
	56	43,052	37,740	
PWM[c]	0	67,115	74,705	1,749
	28	38,420	38,913	
	56	41,285	46,853	

[a]Peripheral blood lymphocytes were stimulated with the following mitogens: concavalin A (ConA, 10 ug/mL, Sigma Chemical Co.), phytohemagglutinin-P (PHA, 10ug/mL Sigma Chemical Co) , and pokeweed mitogen (PWM, 10 ug/mL, Sigma Chemical Co).
[b]Response analyzed over time (days post weaning) as repeated measure. Nonstimulated blastogenesis rate has been subtracted from stimulated blastogenesis rates for the corresponding period. Least square means are reported.
[c]Days post weaning effect, $P<.05$.
[d]FA*day effect, $P\leq .05$.

Table 7. Effect of gestational folic acid supplementation of 0 or 8 mg FA/sow/d on offspring growth and efficiency of feed utilization postweaning[a].

Criteria	Days post Weaning	Sow FA supplementation, mg/kg 0	8	SEM
No. of litters		18	17	
Pig weight, kg	0	3.29	3.17	.10
	28	9.12	8.40	.30
	56	27.98	26.61	.49
Weight gain, kg/d	0 to 28	0.21	0.19	.01
	28 to 56	0.67	0.65	.01
Feed intake, kg/d	0 to 28	0.30	0.27	.01
	28 to 56	0.67	0.65	.01
Gain:feed ratio	0 to 28	0.70	0.68	.01
	28 to 56	0.58	0.59	.01

[a]Least square means reported.

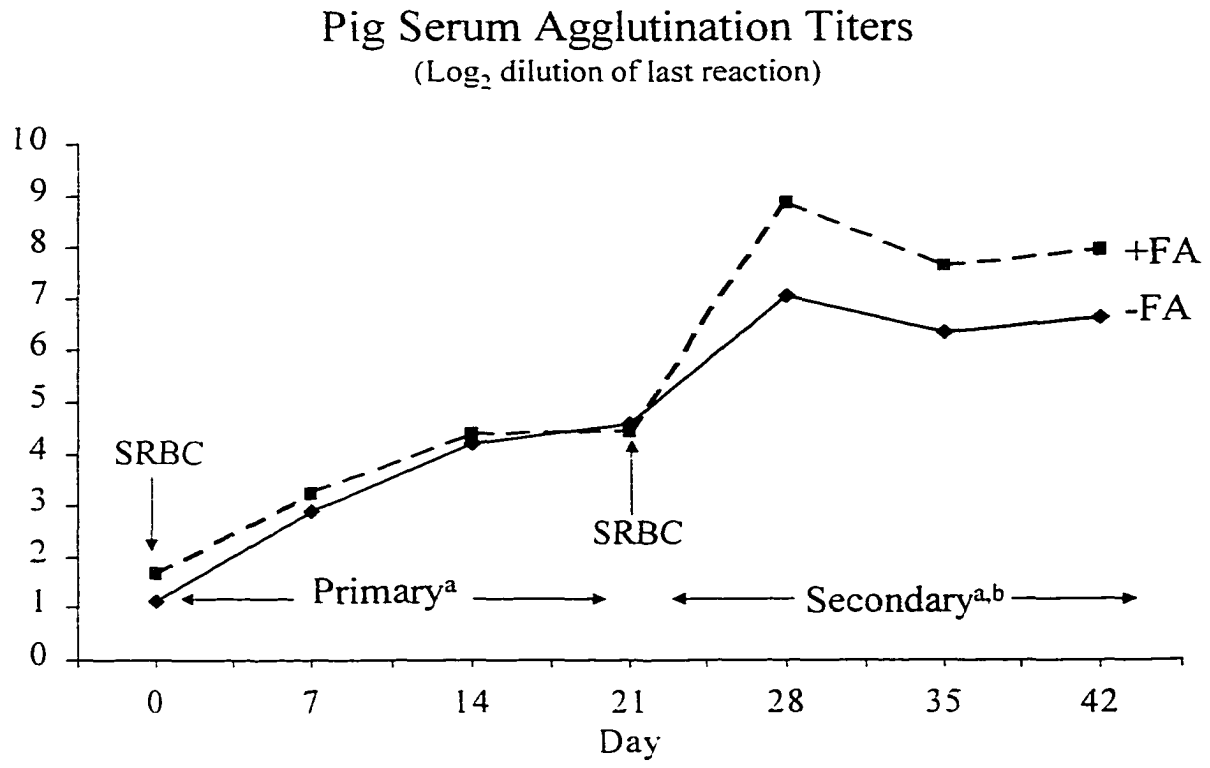

Figure 2. Effect of gestational folic acid (FA) supplementation of 0 (-FA) or 8 (+FA) mg FA/d on pig serum agglutination titers following a primary and secondary sheep red blood cell (SRBC) challenge. Least square means are presented. Agglutination titers during the primary and secondary response period were analyzed separately as repeated measures using the initial agglutination titers for each period as a covariate. The SEM of the primary and secondary responses are .08 and .09, respectively.

[a]Day effect within primary and secondary response period, $P<.01$.

[b]FA effect in secondary response period, $P<.05$.

secondary response, which is typically more specific, rapid, and of a greater magnitude, is enhanced by gestational FA supplementation.

Gestational FA supplementation did not affect pig daily gain, feed intake, or gain:feed ratio during the primary response period (P>.10), but did decrease (P<.05) the daily gain of pigs during the secondary response period (Table 8). It is possible, that had the antigen presented to the pigs been of greater magnitude or of an infectious type, an improvement in performance of pigs from FA supplemented dams might have been observed due to an enhanced capacity to response to and eliminate the immune challenge. It is possible that the offspring of the unsupplemented dams would have required a greater time to recover and would have experienced a prolonged reduction in growth under these conditions.

Implications

Numerous environmental factors, such as sow nutrient supply, influence whether the offspring will survive and what proportion of the offspring's genetic capacity for growth will be expressed. Folic acid is a critical nutrients for prenatal development of the offspring's immune system. In this experiment, the dietary folic acid regimen of sows not only influenced the sow's serum folic acid concentration, but also impacted the postnatal immune response of the offspring, as demonstrated by an alteration in peripheral lymphocyte characteristics, lymphocyte blastogenesis rate, and immune response to a sheep red blood cell challenge. These results would imply that gestational folic acid supplementation could impact the offspring's immune capacity and even survivability when exposed to an immune challenge postnataly.

Table 8. Effect of gestational folic acid supplementation of 0 or 8 mg/sow/d on pig growth performance during the primary (P) and secondary (S) response[a] to a sheep red blood cell (SRBC) challenge.

Criteria	Response Period	Sow FA Supplementation, mg/kg 0	8	SEM
No. of litters	P	12	12	
	S	11	12	
Initial pig weight, kg	P	39.93	40.25	.614
	S	59.47	58.73	.769
Weight gain, kg/d	P	0.91	0.88	.014
	S[b]	0.87	0.78	.015
Feed intake, kg/d	P	2.28	2.27	.047
	S	2.51	2.36	.052
Gain:feed ratio	P	0.40	0.39	.006
	S	0.35	0.33	.004

[a]Primary and secondary response periods correspond to d 0-21 and d 21-42 post initial challenge, respectively.
[b]FA effect, $P<.05$.

Appendix 1. Flow cytometry antibody usage.

Primary Antibody	Final Dilution	Secondary Antibody	Final Dilution
IgG2a anti-CD2	1:150	Anti-IgG2a-PE	1:1250
IgG2a anti-CD4	1:150	Anti-IgG2a-PE	1:1250
IgG2b anti-CD8	1:150	Anti-IgG2b-FITC	1:500
IgG2b anti-IgM	1:75	Anti-IgG2b-FITC	1:500
Isotype Control			
IgG2a	1:150		
IgG2b	1:75		

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CHAPTER 3. THE EFFECTS OF GESTATIONAL FOLIC ACID SUPPLEMENTATION OF SOWS ON OFFSPRING MUSCLE DEVELOPMENT AND POSTNATAL GROWTH RESPONSE

A paper to be submitted to Journal of Animal Science
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Abstract

Primiparous sows (113±27 kg BW) from nineteen pairs of littermates were penned individually and fed daily 1.9 kg of a basal, low folic acid (FA) diet supplemented with 0 or 8 mg folic acid (FA) from coitus to parturition. All sows were fed the basal diet for 112 days prior to breeding to minimize the sows initial body FA stores. At 5±2 d post-insemination, ovulation rate was determined via laparotomy. At birth, four pigs from each litter were randomly chosen based on the proximity of their bodyweight to the mean weight of the litter. Two pigs were sacrificed at birth, prior to suckling, in order to determine the effect of FA on body composition at birth, and two pigs were weaned at 10±3 d, penned individually and self-fed a nutritionally adequate diet to a BW of 107±2.5 kg to determine the effect of FA supplementation on growth performance and body composition at market. Folic acid supplementation in sows during gestation resulted in an elevated ($P<.01$) concentration of serum FA. However, FA supplementation did not affect litter birth weight, dissected litter muscle, fat, and bone weights, or litter DNA and protein content of the muscle at birth, but did increase ($P<.10$) litter whole body DNA (30.45 vs 37.85 mg) and protein (1.04 vs 1.28 kg) content at birth. FA supplementation of the sow also did not affect the offspring's body weight gain, feed intake or gain:feed ratio from weaning to 107 kg. Based on these data, a

dietary folic acid regimen of .3 ppm (.53 mg/d) during pregnancy supports normal muscle growth in pigs pre- and postnatally.

Key words: Pigs, Folic Acid, Muscle, and Growth

Introduction

Economically important goals in pork production include the production of healthy, viable offspring that grow muscle tissue rapidly and produce highly muscled carcasses. Numerous environmental factors, including the dam's nutritional status, influence whether the offspring will survive and what proportion of an offspring's genetic capacity for muscle growth will be expressed both pre- and postnatally.

Muscle growth occurs via two processes: hyperplasia, increased cell number, and hypertrophy, increased cell size. The muscle cell or fiber number in swine is determined by the amount of fetal hyperplastic growth that occurs from conception thru day 70 of gestation (Wigmore and Stickland, 1983a). Therefore, an adequate supply of precursor DNA material is necessary for the hyperplastic growth of fetal muscle cells. Subsequent muscle growth is primarily due to hypertrophy, resulting from increased protein synthesis in each muscle cell or fiber (Swatland, 1973). The quantity of fetal DNA, RNA, and protein in a muscle influences birth weight (Wigmore and Stickland, 1983b), and postnatal growth (Dwyer et al., 1994).

Transplacental passage of nucleotides, nucleosides, and bases is possible in rats (Hayashi and Garvey, 1968). But since the placental structure of the pig is less permeable than that of the rat, mouse, and human, it is hypothesized that placental transfer of DNA

precursors in swine is minimal, and therefore these substances must be synthesized in the fetus.

Folic acid (FA) has a fundamental role in *de novo* synthesis of DNA (Herbert and Das, 1976). Therefore, the amount of FA available to the dam may influence the amount of hyperplastic growth (ie. muscle cell growth) that occurs in fetal tissues. Serum FA concentration in gravid sows decline to minimal levels during mid-gestation, which corresponds with the period of critical hyperplastic muscle growth (Matte et al., 1984; Wigmore and Stickland, 1983a). The rate of FA catabolism has also been shown to increase during pregnancy in the rat (McNulty et al., 1993). These findings support the hypothesis that the FA requirement is increased during pregnancy, assumably to support the rapid rate of DNA synthesis occurring in both fetal and maternal tissues.

The objective of this experiment was to determine the influence of gestational FA supplementation of sows on muscle development and subsequent growth performance of the offspring.

Materials and Methods

Treatments

Primiparous sows were fed daily 1.9 kg of a basal, low FA diet (0.28 mg/kg, Table 1) supplemented with 0 or 8 mg FA from coitus through parturition. This dietary regimen provided a daily intake of metabolizable energy, amino acids, minerals, and vitamins (except FA) that met or exceeded the estimated nutrient needs (NRC, 1988) of the gravid sow.

Table 1. Composition of basal diet

Ingredient	% of Diet
Corn, ground	91.58
Casein	3.49
L-Lysine-HCl, 98.5%	0.12
L-Threonine	0.04
Tryptophan/lysine blend[a]	0.266
Dicalcium phosphate	2.79
Limestone	0.10
Salt	0.50
Potassium sulfate	0.50
Trace mineral mix[b]	0.11
Choline chloride, 60%	0.30
Vitamin mix[c]	0.10
Chloratetracycline mix[d]	0.10

[a]ADM tryptosine[TM] 15/70 contained 55.3% L-Lysine, 15% L-tryptophan, 1.75% methionine, 0.5% valine, and 0.15% threonine.

[b]Provided per kg of diet: 10.5 mg Cu, 105 mg Fe, 36 mg Mn, 90 mg Zn, 0.12 mg I, and .30 mg Se.

[c]Provided per kg of diet: 0.75 mg biotin, 30 mg niacin, 44 mg pantothenic acid, 14 mg riboflavin, 1.0 mg pyridoxine, 1.0 mg thiamine, 70 IU vitamin E, 16,000 IU vitamin A, 800 ICU vitamin D_3, 2 mg vitamin K, and 60 ug vitamin B_{12}.

[d]Provides 110 ppm chloratetracycline per kg of diet.

Sow management and procedures

Nineteen littermate pairs of crossbred primiparous sows of Landrace, Yorkshire, Duroc, and Hampshire ancestry were evaluated. In an effort to ensure minimal body FA stores, all sows were fed 1.9 kg/day of the basal diet (0.28 mg FA/kg diet) for a total of 112 days prebreeding (Tables 1 and 2). Beginning on depletion d 98, sows were synchronized by feeding daily 14 mg altrenogest (Regu-Mate®, Roussel UCLAF, Agro-veterinary Division) per sow for 14 days. Sows were mated and managed during pregnancy and lactation as described by Grieshop et al. (1999). During lactation, sows were fed a corn-soybean meal diet formulated to meet or exceed all NRC recommendations for a lactating sow (NRC, 1988).

Folic acid status monitoring

Sow serum FA concentrations were determined weekly in 15 representative sows during the 112 d depletion period (with the exception of depletion d 105), and weekly in all

Table 2. Dietary feeding regimens.

Recipient	Stage of Development	Days	Diet	Analyze FA[a], mg/kg diet	Feed offered
Sow	Prebreeding	112	Basal (-FA)	.28	1.9 kg/d
Sow	Pregnancy	114	Basal (-FA)	.28	1.9 kg/d
			Basal (+FA)	4.36	1.9 kg/d
Sow	Lactation	11	Corn/SBM	1.18	Ad lib
Pig	Nursery	56	Corn/SBM/Whey	2.36	Ad lib
Pig	Grower[b]		Corn/SBM	2.07	Ad lib

[a]Folic acid (FA) analyzed via Quantaphase II B12/Folate Radioassay, Bio-Rad.
[b]Pigs were fed the grower diet from 60±3 d postweaning to time of slaughter at 110±5 kg.

sows from d 28 to 105 of pregnancy via radioimmunoassay (Bio-Rad Quantaphase B12-Folic Acid kit), as described by Grieshop et al. (1999). Pig serum FA concentration (prior to suckling) also was determined in one pig per litter at birth. Twenty-four hour urine output was collected weekly from 15 representative sows during the 112 d depletion period and from all sows on dietary treatments from d 28 to 105 of pregnancy via Foley catheters for subsequent determination of folic acid metabolite concentration.

Offspring management procedures

At birth, pigs were processed and managed as described by Grieshop et al. (1999). At 10±3 d of age, two pigs (barrows if available) per litter, previously chosen based on the proximity of their birth weight to the mean birth weight of the litter, were treated with ivermectim (Ivomec, Merck-AG Vet, Rahway, NJ), weaned, and transported to nursery rooms isolated from the original sow complex. Only if barrows of the appropriate weight were not available, gilts were used (<15% of pigs used). Each pig was individually penned on a slotted floor in .48 x 1.22 m pens and allowed to consume feed and water ad lib. Pigs were fed a commercial milk based diet (Soweena® Litter Bites #1, Merrick's, Middleton, WI) for the initial 7 d and then a fortified corn-soybean meal-whey diet (Table 2). The corn-soybean meal-whey diet was formulated to meet or exceed the NRC (1988) estimated nutrient requirement for a pig fed from 5 to 20 kg BW. At d 60±3 d postweaning, all pigs were moved to an isolated growth facility and individually penned on slotted floors in 0.6 m x 2.1 m pens. Pigs were fed a fortified corn-soybean meal diet formulated to meet or

exceeded all NRC (1988) estimated nutrient requirements for pigs 20 to 110 kg BW (Table 2). Body weights and feed consumption were determined weekly.

Assessment of pre- and postnatal muscle development

At birth, two pigs from each litter were selected based on the proximity of their weight to the mean weight of the previous pigs farrowed and sacrificed prior to colostrum consumption, via a jugular injection followed by intracardial injection of sodium pentobarbital. These pigs were exsanguinated and frozen for later dissection.

The whole bodies (with blood removed) of pigs sacrificed at birth were thawed at a later time. Three muscles (longissimus dorsi, rectus femoris, and semimembranosus) and two organs (thymus and spleen) were isolated, weighed, and refrozen at -20^{0}C for subsequent analysis. The remainder of the body was physically dissected into components of muscle, fat, bone, viscera, head, feet, and tail, and each component was weighed. The dissected muscle was sampled and then all components were combined and ground in liquid nitrogen and stored at -20^{0}C for subsequent analysis. Whole body weight losses which occurred during freezer storage and dissection were assumed to be due to water associated with muscle. Therefore, the muscle content of the whole body was corrected for this loss. DNA content of the whole body, dissected muscle, and each of the individual muscles were determined by fluorometric analysis (Labarca and Paigen, 1980). Protein content of the whole body and longissimus muscle was determined by Kjehdahl nitrogen analysis (AOAC, 1984). Composition of the total litter at birth was estimated by multiplying the mean concentration of each component present in the two pigs per litter sacrificed at birth by the

litter birth weight.

When pigs reached a BW of 110±5 kg, they were transported 3 km from the growth facility to the Iowa State University Meat Lab. Pigs were then weighed, electrically stunned, and killed via exanguination. Following scalding and removal of the head, the thymus, spleen, heart-lungs, liver, kidneys, gastrointestinal tract (with digesta), reproductive tract, and jowl trim were removed and weighed. Carcasses were then split in half, reweighed, and chilled for 20 to 24 hours at -2° C.

After chilling, cold carcass weight, longissimus muscle area at the tenth rib, backfat thickness at the tenth rib, midline backfat thickness at the first rib, last rib, and last lumbar vertebrae, and carcass length were taken on each half of the carcass. Three muscles (longissimus dorsi, rectus femoris, and semimembranosus) were then isolated from the right side of the carcass, weighed, and frozen for subsequent analysis of DNA (Labarca and Paigen, 1980) and protein content (AOAC, 1984).

The animal care procedures employed in this study were approved by the Iowa State University Committee on Animal Care.

Statistical analysis

The study was analyzed as a randomized completed block design with the sow considered the experimental unit and the sow or boar littermate-pair the block. Data collected from multiple offspring from the same litter were averaged for analysis. Data were analyzed by analysis of variance techniques using general linear model (GLM) procedure of SAS (1996). The error term used to test the effect of gestational FA supplementation was the FA

by block interaction.

Pig BW at weaning was used as a covariate in the analysis of the offspring BW gain, feed intake, and gain:feed data. Pig BW at slaughter was used as a covariate in the analyses of carcass traits, organ weights, and muscle characteristics of pigs killed at 110 kg BW. Least square means are reported unless stated otherwise. The relationships between sow serum FA concentration and growth performance parameters were analyzed using the linear regression procedure of SAS (1996).

Results and Discussion

Feeding the basal, low FA diet during the 112 d prebreeding depletion period resulted in a progressive reduction ($P<.05$) in the sow serum FA concentration (Figure 1). Based on these data, the goal of minimizing body FA prior to the initiation of the experiment was achieved.

Dietary supplementation of 8 mg/sow/d during pregnancy increased ($P<.05$) serum FA concentration in sows (Figure 1). Sow serum FA concentrations declined to minimum levels at d 70 of pregnancy in both the basal and supplemented groups (21.5 and 47.5 ng/ml, respectively). The serum FA concentration then increased thru d 84 or 91 and then again declined preparturition. The initial decline in sow serum FA from day 28 to 70 of pregnancy corresponds to the period of hyperplastic muscle fiber growth in the fetus (Wigmore and Stickland, 1983a), while the latter decrease corresponds to the period of initial colostrum synthesis (Kensinger et al., 1982).

A similar reduction in sow serum FA concentration during pregnancy was observed

by Matte et al. (1984) who fed sows a total of 1.2 mg FA/d., although the severity of reduction was less (minimal level of 40 ng FA/ml) than that observed in the unsupplemented sows (22 ng/ml) in this experiment. Reductions in blood FA levels have also been reported during mid pregnancy in lactating dairy cows (Girard et al., 1989). Low gestational serum folic acid levels have been associated with low birth weight, greater incidences of fetal–growth retardation (Tamura et al., 1997), and increased pre-term delivery (Scholl et al., 1996) in humans.

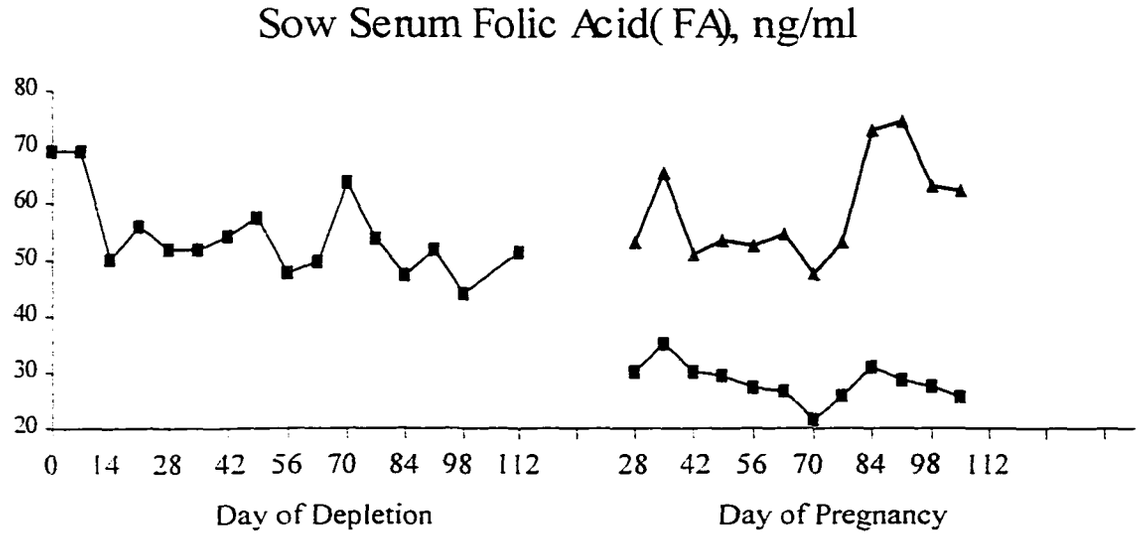

Figure 1. Effect of dietary gestational folic acid (FA) supplementation of 0 (■) or 8 (▲) mg FA/kg diet on serum FA concentration in sows during a 112 d depletion period and 77 d phase of pregnancy. Least square means represent the values of 15 and 39 sows during the depletion and pregnancy stages, respectively. Sow serum FA concentration during pregnancy was influenced ($P<.01$) by gestational FA supplementation.

The number of pigs born (8.10 vs 8.15) and litter birth weight (10.49 vs 11.52 kg) were not altered by gestational FA supplementation. These results are contrary to those of Thaler et al. (1989) and Matte et al. (1984) who observed increases in litter size due to folic acid supplementation, but in agreement with results of Matte et al. (1993), Easter et al. (1983), and Harper et al. (1994) who did not observed any effect of supplementing folic acid on litter size. The proportion of ova that were fertilized, survived, and subsequently farrowed in this experiment were less than anticipated. Factors that likely contributed to the reduced litter size include: laparotomies performed on d 5 post coitus and the seroconversion of some sows for pseudorabies during the 112 d depletion period of this study. Unfortunately, this reduction in number of fetus during gestation, presumable reduced the folic acid demand during pregnancy.

Gestational FA supplementation resulted ($P<.10$) in greater whole body DNA and protein content of the litter at birth (Table 3), however gestational folic acid supplementation did not alter weight and DNA content of the longissimus dorsi, semimembranosus, or rectus femoris muscles (Table 3). These particular muscles where chosen due to their temporal diversity in fetal development (semimembranosus and rectus femoris muscles developing during mid gestation, and longissimus muscle during mid to late gestation, Swatland, 1973). The fact that gestational FA supplementation did not affect development of any of these muscles would imply that at no point during gestation of these sows was folic acid the limiting factor in development of these fetal muscles. It is possible that the increase in whole body DNA and protein content of the litter due to gestational FA supplementation was due to a numerical increase in litter weight. Gestational folic acid supplementation also did not

Table 3. Effect of gestational folic acid (FA) supplementation of 0 or 8 mg FA/sow/d on total litter body and muscle DNA and protein content at birth (presuckle)[ab] .

Tissue	Criteria	Sow FA supplementation, mg/sow/d		SEM
		0	8	
Whole body	DNA, g[c]	30.45	37.85	1.80
	Protein, kg[c]	1.04	1.28	0.06
Total Muscle	DNA, g	10.54	12.44	0.75
Rectus femoris	Wt, g	29.65	30.39	1.66
	DNA, mg	115.17	109.83	5.96
Semimembranosus	Wt, g	40.36	47.89	2.36
	DNA, mg	144.49	172.08	9.49
Longissimus dorsi	Wt, g	99.20	120.94	7.65
	DNA, mg	361.21	420.41	27.06
	Protein, g	10.15	12.53	0.67

[a]Litter muscle DNA and protein content were calculated by multiplying the concentration of each muscle component in the individual pigs sacrificed at birth by the litter birth weight.
[b]Values reported are least square means.
[c]Differences due to folic acid supplementation $P<.10$.

influence the total weight of muscle, fat, and bone at birth (Table 4). Utilizing the means weight of the muscle, fat, and bone at birth, and the mean total litter weight at birth, the percent of total litter body weight due to each of these components was calculated. The percent of litter body weight due to each component was: muscle 28.9 and 29.3%, fat 0.5 and 0.5%, and bone 13.2 and 12.8% for offspring from unsupplemented and FA supplemented sows, respectively. This information supports the conclusion that gestational FA is not limiting in fetal tissue development, and therefore does not affect tissue distribution of the offspring at birth.

Gestational FA supplementation did not alter individual muscle weight or DNA content of the offspring when slaughtered at a BW of 107 kg (Table 5), nor did it affect the offspring's carcass traits or organ weights at this weight (Table 6). Since it was previously demonstrated that gestational FA supplementation did not affect tissue distribution or muscle DNA content at birth, and it is known that the muscle cell or fiber number in swine is determined by the amount of hyperplastic growth that occurs prenatally (Wigmore and Stickland, 1983a), these results were not unexpected.

Gestational FA supplementation of the dam did not affect average pig weight at weaning (3.16+0.57 kg). Gestational FA supplementation also did not alter pig daily BW gain, feed intake, or feed:gain ratio from weaning thru slaughter (106.6+2.5 kg BW) (Table 7). Daily muscle gain during the entire period of birth to slaughter also was not affected by gestational FA supplementation (Table 7). Again, since FA supplementation did not effect offspring muscle or whole body composition at birth, these results are not unexpected.

Table 4. Effect of gestational folic acid (FA) supplementation of 0 or 8 mg FA/sow/d on total litter tissue weights at birth (presuckle)[ab].

Tissue	Sow FA supplementation, mg/sow/d		SEM
	0	8	
Number of litters	17	19	
Total litter weight, kg	9.92	11.85	0.64
Carcass tissue weights			
Muscle, kg	2.87	3.47	0.18
Fat, kg	0.05	0.06	0.01
Bone, kg	1.31	1.52	0.08
Skin, kg	0.87	1.03	0.06
Visceral organs, kg[c]	1.40	1.83	0.08
Head, kg	1.80	2.17	0.11
Feet and tail, kg	1.09	1.28	0.07

[a]Litter tissue weights were calculated by multiplying the concentration of each tissue in the individual pigs sacrificed by the litter birth weight.
[b]Values reported are least square means.
[c]Difference due to folic acid supplementation, $P<.05$.

Table 5. Effect of gestational folic acid (FA) supplementation of 0 or 8 mg FA/sow/d on offspring individual muscle characteristics at a BW of 107 kg[a].

Criteria	Sow FA Supplementation, mg/sow/d		SEM
	0	8	
Number of litters	18	17	
Longissimus dorsi			
Weight, kg	2.39	2.28	0.04
DNA concentration, mg/g	1.29	1.30	0.02
DNA content, g	3.05	2.95	0.07
Semimembranosus			
Weight, kg	0.74	0.79	0.02
DNA, mg/g	1.52	1.51	0.02
DNA content, g	1.11	1.20	0.04
Rectus femoris			
Weight, kg	0.40	0.40	0.01
DNA, mg/g	1.40	1.39	0.02
DNA content, g	0.55	0.55	0.01

[a]Least square means reported. Data adjusted for pig BW.

Table 6. Effect of gestational folic acid (FA) supplementation of 0 or 8 mg FA/sow/d on offspring carcass traits and organ weights at 107 kg BW.[a]

Criteria	Sow FA Supplementation, mg/sow/d		SEM
	0	8	
Number of litters	18	17	
Pig weight, kg	107.02	106.95	0.55
Hot carcass weight, kg	80.74	80.34	0.27
Hot carcass yield, %	75.56	75.16	0.26
Average midline backfat, cm[b]	2.92	3.04	0.06
Tenth rib backfat, cm	2.64	2.79	0.10
Loin eye area at 10th rib, sq cm	36.92	36.46	0.71
Carcass length, cm	80.16	79.29	0.21
Estimated carcass muscle, %[c]	56.91	56.25	0.49
Organ weights, kg			
Heart and lungs	1.55	1.55	0.03
Liver	1.50	1.56	0.02
Leaf fat	1.44	1.56	0.05
Kidneys	0.32	0.33	.005
Thymus	0.11	0.10	.005
Spleen	0.15	0.14	.003
Gastrointestinal tract	8.12	8.38	0.13
Head	5.91	6.00	0.06
Reproductive tract[d]	0.24	0.28	0.02

[a]Least square means are reported. Pig weight immediately preslaughter was used as a covariate in data analysis.

[b]Average of midline backfat measurements at the first rib, last rib, and last lumbar vertebrae.

[c]Estimated carcass muscle calculated as: {[((2+(hot carcass weight, lb*.45)+(loin eye area, sq in.*5)-(tenth rib backfat, in.*.9))*.4545]/hot carcass weight, kg}*100.

[d]Tracts were not collected from three and two pigs in the 0 and 8 mg/d FA treatment groups, respectively.

Table 7. Effect of gestational folic acid supplementation of 0 or 8 mg FA/d on offspring growth and efficiency of feed utilization postweaning[a].

Criteria	Day post Weaning	Sow FA supplementation, mg/sow/d 0	8	SEM
No. of litters	0	19	19	
	56	19	19	
	Slaughter	18	17	
Pig weight, kg	0	3.05	3.33	.09
	56	28.5	30.0	.56
	Slaughter	106.3	107.0	.60
BW gain, kg/d	0 to 56	0.47	0.49	.01
	56 to slaughter	0.84	0.88	.02
Feed intake, kg/d	0 to 56	0.77	0.81	.01
	56-slaughter	2.59	2.71	.05
Gain:feed ratio	0 to 56	0.61	0.61	.01
	56-slaughter	0.33	0.32	.005
Muscle gain, kg/d[b]	Birth-slaughter	0.28	0.29	.01

[a]Least square means reported. Pig BW at weaning was used as a covariate.
[b]Pig BW at birth was used as a covariate.

Implications

Numerous environmental factors, such as gestational dietary folic acid, influence whether the offspring will survive and what proportion of the offspring's genetic capacity for growth will be expressed. In this experiment, dietary folic acid supplementation of sows during gestation did influences the sow's serum folic acid concentration, but did not alter offspring prenatal or postnatal muscle development or postnatal growth performance. These results imply that gestational folic acid is not limiting in prenatal development of the offspring of sows under these experimental conditions.

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CHAPTER 4. GENERAL DISCUSSION

Numerous environmental factors, including the dam's nutritional status, influence whether the offspring will survive and what proportion of the offspring's genetic capacity for growth will be realized both pre- and postnatally in swine. Critical factors for swine production success include not only the number of offspring born alive, but also the tissue composition of the offspring, the growth potential of the offspring, and the capability of the offspring the successfully mount an immune response to an antigen challenge.

In the study described in the previous two chapters, nineteen pairs of littermate primiparous sows were fed daily 1.9 kg of a basal, low folic acid diet (.28 mg FA/kg) supplemented with 0 or 8 mg folic acid (FA) from coitus to parturition in an effort to determine the effects of gestational FA supplementation on the offspring immune organ and muscle development, and postnatal immune and growth response. Since factors such as dietary regimen prior to the initiation of the study and previous FA demands may have impacted the sows' body FA stores, all sows were fed the basal diet for 112 days prior to mating to minimize and equalize body folic acid stores.

During pregnancy, serum FA concentration declined thru mid-pregnancy (d 70) then increased to d 84 or 91, and finally declined as parturition approached. It is hypothesized that the decline in serum FA concentration during mid-pregnancy was due to a high demand for FA for the synthesis of DNA precursor nucleotides necessary for the developing fetal tissues. Folic acid supplementation elevated sow serum FA throughout gestation and resulted in a greater serum concentration of FA in the offspring at birth.

Gestational FA supplementation did not affect pigs born/litter nor litter birth weight in this study. Colostral transfer of immunoglobulins to the pig is critical since transplacental passage of immunoglobulins does not occur in swine. Gestational FA supplementation did not alter immunoglobulin (IgA, IgG) concentrations in sow serum on d105 of pregnancy or in colostral whey or pig serum at parturition. It was concluded from these observations that sow immunoglobulin synthesis and colostral transfer of immunoglobulins from the sow to the offspring was not affected by gestational FA supplementation.

The effect of FA supplementation on offspring immune organ development and immune capacity was assessed by characterization of the immune status at birth and exposure of the offspring to both a general and a specific immune challenge postweaning. Gestational FA supplementation did not effect the development of the immune organs in utero, as assessed by pig thymus and spleen weight, DNA, and protein content at birth. Although, gestational FA supplementation did alter offspring peripheral blood lymphocyte characteristics postweaning when the pig was exposed to a general immune challenge. During this challenge, the percent of CD8 positive lymphocytes increased from weaning thru d 14, then declined by d 21. The magnitude of change in CD8 positive peripheral blood lymphocytes tended to be affected by gestational FA supplementation. A significant FA by day interaction existed for the percent of CD2 positive peripheral blood lymphocytes during the general immune challenge. Gestational FA supplementation also increased offspring lymphocyte blastogenesis rate upon stimulation with PHA at d 0 but not d 21 postweaning, also resulting in a FA by day interaction. Although differences appeared to existed in the immune capacity of the offspring, as determined by the parameters described, no differences

in performance, body weight gain, feed intake, and gain:feed ratio of the offspring were detected during this period.

One key finding of this experiment was that FA supplementation of the dam resulted in greater serum agglutination titers in offspring following a secondary sheep red blood cell challenge. Since pigs are generally vaccinated early in life against the prevalent antigens in the herd, these results would imply that pigs from FA supplemented dams would be more capable of mounting a secondary immune response to these antigens upon exposure from their environment.

The effect of FA supplementation on offspring muscle development and postnatal growth was assessed by characterization of the tissue distribution at birth, monitoring the postnatal growth rate and efficiency, and characterization of the tissue distribution at slaughter. Folic acid supplementation did not affect litter birth weight, dissected litter muscle, fat or bone weights, or muscle characteristics at birth. But, FA supplementation did increase litter whole body DNA and protein content at birth. Folic acid supplementation of the sow did not affect the offspring's postnatal body weight gain, feed intake, gain:feed ratio, or carcass characteristics at a BW of 107 kg.

Based on the data collected in this study, FA status of the gravid dam influenced postnatal immune response of the offspring and, in the event of a secondary exposure to an antigen, offspring from FA supplemented dams would have a greater capacity to mount an immune response. But, FA supplementation of .53 mg/d did not effect pre- or postnatal muscle growth and did not impact carcass composition of the offspring at slaughter.