# EFFECT OF FEEDING SUPPLEMENTAL VITAMIN $B_6$ ON THE REPRODUCTIVE PERFORMANCE AND NUTRIENT METABOLISM IN LEAN GENOTYPE SOWS

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by

Tracy Ellen Noelle Knights

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# EFFECT OF FEEDING SUPPLEMENTAL VITAMIN $B_6$ ON THE REPRODUCTIVE PERFORMANCE AND NUTRIENT METABOLISM IN LEAN GENOTYPE SOWS

BY

TRACY ELLEN NOELLE KNIGHTS

A Thesis/Practicum submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

## Abstract

KNIGHTS, TRACY ELLEN NOELLE. M.Sc., The University of Manitoba, October 1996. Effect of Feeding Supplemental Vitamin $B_6$ on the Reproductive Performance and Nutrient Metabolism in Lean Genotype Sows. Major Professor; Sam K. Baidoo.

Two 2 x 2 x 2 factorial experiments utilizing 181 second parity sows were conducted to determine the effect of feeding supplemental vitamin $B_6$ during the postweaning and gestation periods on the reproductive performance of lean genotype sows. Treatments consisted of two breeds (Hampshire and Yorkshire), two genotypes (control and a line selected for low backfat over six generations), and two levels of vitamin $B_6$ (1 mg and 15 mg/kg of feed). In addition to reproductive performance, a metabolic study was conducted utilizing 48 sows at 55-60 days gestation. Diets consisted of a barley and soybean meal mix with 13% crude protein. Conception rate, number of stillbirths, and mean birth weights of piglets were not affected by the breed, genotype, or level of vitamin $B_6$ in the diet. Vitamin $B_6$ supplemented at 15 mg did significantly ($P<0.05$) decrease the weaning-to-estrus interval by 1 day in second parity sows. Weight changes during the postweaning period were ($P<0.01$) different between breeds, but not between the genotypes or diets. The gestational weight gain differed ($P<0.05$) between genotypes only. Litter size was not different ($P>0.05$) between breeds, genetic lines, or diets. However, sows fed 15 mg of vitamin $B_6$ had slightly larger litters ($P=0.09$) compared to sows fed 1 mg. In the metabolic study, sows fed 15 mg of vitamin $B_6$ had significantly ($P<0.01$) lower dry matter retentions. This was most likely due to a lower

($P<0.01$) dry matter digestibility in these sows. Sows fed 15 mg of vitamin $B_6$ had a 5% increase in nitrogen retention ($P=0.16$) than the control group sows. The slight increase in litter size is most likely the reason for this nitrogen retention increase. Plasma urea nitrogen (PUN) levels did not differ between diets or genotypes. Yorkshire sows had high PUN levels during the last two-thirds of pregnancy compared to Hampshire sows. The higher PUN concentrations indicate decreased amino acid utilization and may explain the significantly lower nitrogen retention in these Yorkshire sows, with genetic potential being the cause. The results of this study indicate that supplementation of vitamin $B_6$ above NAS-NRC (1988) recommendation of 1 mg/kg of feed does not significantly improve reproductive performance or nutrient retention in lean genotype sows. However, the significant decrease in weaning-to-estrus interval and a slight increase in litter size, as well as, slightly improved nitrogen retention may indicate that if economically viable, vitamin $B_6$ supplementation in sow rations could be beneficial to swine production.

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## ABBREVIATIONS

| | |
|---|---|
| ARC | Agricultural Research Council |
| ALP | Alkaline phosphatase |
| Ca | Calcium |
| $\chi^2$ | Chi-square |
| CP | Crude protein |
| DM | Dry matter |
| DNA | Deoxyribonucleic acid |
| EGOT | Erythrocyte glutamate oxaloacetate aminotransferase |
| GABA | $\gamma$-aminobutyric acid |
| HPLC | High performance liquid chromatography |
| MJ | Mega joule |
| NRC | National Research Council |
| P | Phosphorous |
| PA | Pyridoxic acid |
| PL | Pyridoxal |
| PLP | Pyridoxal-5´-phosphate |
| PM | Pyridoxamine |
| PMP | Pyridoxamine-5´-phosphate |
| PN | Pyridoxine |
| PUN | Plasma urea nitrogen |
| THFA | $N_5$, $N_{10}$-methylene tetrahydrofolic acid |

# INTRODUCTION

The role of vitamin $B_6$ in the normal functioning of various enzymes has long been known. Vitamin $B_6$ functions as a cofactor for over 50 enzymes involved in amino acid, carbohydrate, and fat metabolism (McDowell, 1989). In addition, various steroid hormones are believed to be regulated by vitamin $B_6$ (Allgood and Cidlowski, 1991). Bioavailability of vitamin $B_6$ can be influenced by processing, storage, and the type of feed (Baker, 1995). However, it is generally agreed that the vitamin $B_6$ levels in feedstuffs used in diets are sufficient to meet the requirements of many species (Baker, 1995; Easter *et al.*, 1983).

Numerous studies with humans have indicated that during pregnancy vitamin $B_6$ levels in the plasma decrease (Barnard *et al.*, 1987; Cleary *et al.*, 1975; Shane and Contractor, 1975; Hamfelt and Tuvemo, 1972 - as cited by Shane and Contractor, 1980). Supplementation of diets with vitamin $B_6$ does not necessarily restore these blood levels to those of non pregnant females. The onset of estrus after weaning and the maintenance of pregnancy are controlled by various factors including protein and energy intake and hormonal balance. The role of vitamin $B_6$ in several metabolic pathways indicates it's importance during estrus and gestation. In addition, vitamin $B_6$ has been found to be important in fetal development, the central nervous system in particular (Groziak and Kirksey, 1987; Wasynczuk *et al.*, 1983; Chang *et al.*, 1981; Roepke and Kirksey, 1979; Morre *et al.*, 1978 *a&b*). Viability scoring of human infants (based on the infants appearance, heart rate, respiration, muscle tone, and reaction to slapping at birth) indicated that supplementation of vitamin $B_6$ during pregnancy improves viability scores,

but not necessarily the viability of the infant (Schuster *et al.*, 1984). Other studies with humans, rats, and mice showed no significant increases in fetal weights or litter size when mothers were supplemented with vitamin $B_6$ (Kirchgessner *et al.*, 1985; Cheney and Beaton, 1965).

There is scarcity of data available on the effect of supplemental vitamin $B_6$ on reproduction in the sow. The increased requirement for amino acids by the lean genotypes may increase the requirements for vitamin $B_6$. Past studies have either begun supplementation at 2 months gestation (Ritchie *et al.*, 1960) or at breeding (Wohlbier and Siegel, 1967*a* - as cited by ARC, 1981). Another study by Easter *et al.* (1983) supplemented only 1 ppm of vitamin $B_6$ which is the NAS-NRC (1988) recommended level for pregnant sows.

Amino acid metabolism, particularly tryptophan requires the presence of vitamin $B_6$ as a cofactor (Henderson and Hulse, 1978). Nitrogen retention in growing pigs was found to be maximized when vitamin $B_6$ is supplemented at 2.5 mg/kg of feed (Wohlbier and Siegel, 1967*b*; Kirchgessner and Friesecke, 1961; Moustgaard *et al.*, 1952 - as cited by ARC, 1981). However, no data is available on the amount of vitamin $B_6$ required for optimal nitrogen retention in gestating sows. Information on the vitamin $B_6$ requirements by these lean genotypes is nonexistent.

The objective of this study was to determine the effect of feeding supplemental vitamin $B_6$ above NAS-NRC (1988) recommended levels to gestating lean genotype sows. Reproductive parameters such as weaning-to-estrus interval, postweaning and gestational weight changes, backfat changes, litter size, stillbirths, and birthweights were all

measured. Determination of dry matter, energy, and nitrogen retentions were calculated using nutrient intakes and digestibilities.

# LITERATURE REVIEW

## History of Vitamin $B_6$

Vitamin $B_6$, as many other vitamins, was discovered as a result of investigating into a deficiency symptom. Acrodynia, a pink or florid dermatitis was first observed in 1926 in rats fed a diet deficient in riboflavin. In 1932, Ohdake isolated a compound from rice polishings with the molecular formula $C_3H_{11}O_3N \cdot HCl$, but failed to recognize its future role as a vitamin. Two years later, in 1936, Gyorgy established the difference between the "rat pellagra preventative factor" and riboflavin. The new vitamin was called $B_6$. Gyorgy defined the vitamin as part of the B-complex curative of a specific dermatitis in young rats on a B-complex- free diet supplemented only by thiamin and riboflavin (Rosenberg, 1945 - as cited by Brin, 1978).

Several groups of researchers began the process of isolating the pure crystalline form of vitamin $B_6$. In 1938, five different groups independently announced the isolation of vitamin $B_6$ (Rosenberg, 1945 - as cited by Brin, 1978) Chemical structure and synthesis of vitamin $B_6$ occurred one year later in 1939. This same year, Gyorgy named the vitamin pyridoxine based on its structure (Rosenberg, 1945 - as cited by Brin, 1978). The term vitamin $B_6$ is generally used since there are several forms of the vitamin. However, this generic description refers to those vitamers exhibiting qualitatively the biological activity of pyridoxine.

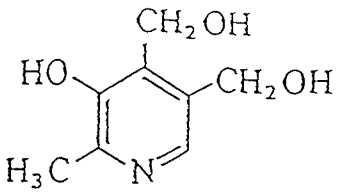
## Physical and Chemical Properties of Vitamin $B_6$

There are six forms of vitamin $B_6$ involved in metabolism. These forms called vitamers include an alcohol (pyridoxine), an aldehyde (pyridoxal), an amine (pyridoxamine), and their 5' phosphates (Figure 1). In addition, the excretory form of the vitamin which has been oxidized is identified as 4-pyridoxic acid. With the exception of 4-pyridoxic acid, these vitamers are metabolically interconvertible (Bender, 1992).

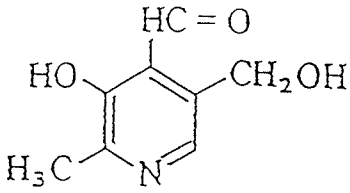
Each vitamer is chemically different and therefore, differs in various properties. The ultraviolet absorption spectra of the vitamers vary significantly with the pH of the solution (Harris *et al.*, 1968 - as cited by Brin, 1978). Each vitamer is converted to a variety of aqueous ionic forms depending on pH and other physical factors (Snell, 1963 - as cited by Brin, 1978). Generally, the three vitamers and their phosphate forms are white and crystalline, and soluble in water and alcohol, but often insoluble in ether. Aqueous solutions of these forms are often unstable and deteriorate in light and heat (Harris *et al.*, 1968; Snell, 1963 - as cited by Brin, 1978).

## Analytical Techniques

The methods of analysis include microbiological, colorimetric, gas chromatographic and ion-exchange chromatographic procedures (LeKlem and Reynolds, 1981). Ion-exchange chromatography using fluorometric compounds requires large serum samples and all of the above procedures are time consuming (Chauhan and Dakshinamurti, 1981 - as cited by Sharma and Dakshinamurti, 1992). They were also found to be less sensitive than high performance liquid chromatography (HPLC).



Pyridoxine (PN)



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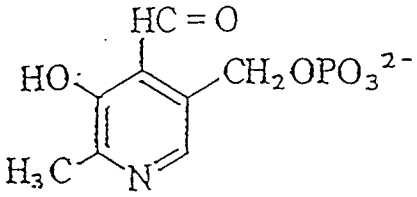
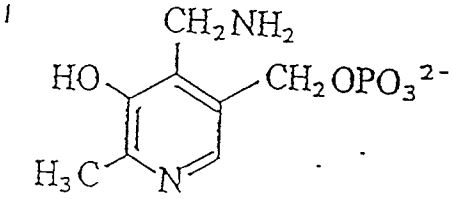
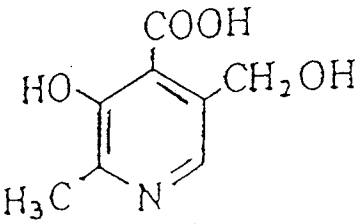


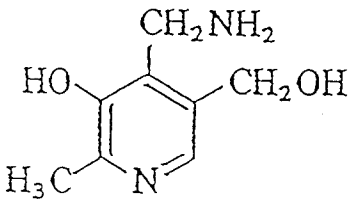
Pyridoxal-5'-Phosphate (PLP)



Pyridoxamine-5'-Phosphate (PMP)



4-Pyridoxic Acid (PA)



Pyridoxamine (PM)

**Figure 1. Structures of Vitamin B6 Nomenclature (Adapted from Brin, 1978).**

Fluorometric detection of vitamin $B_6$ using HPLC has been shown to provide the most accurate and reproducible determination of vitamer concentrations (Vanderslice *et al.*, 1979).

## Mode of Action of Vitamin $B_6$

### Absorption

In feedstuffs, vitamin $B_6$ is bound as enzyme-coenzyme complexes that require hydrolytic release (Storvick and Peters, 1964). In plants, the predominant form is pyridoxine. In animal tissue, both pyridoxal-5'-phosphate and pyridoxamine are common. Absorption of the vitamin occurs mainly in the jejunum and ileum by passive diffusion. Absorption from the colon is small and insignificant (Booth and Brain, 1962 - as cited by Yamada and Tsuji, 1980). Studies with dogs and rats have found that absorption of pyridoxine is rapid and complete (Wolf, 1958; Scudi *et al.*, 1949 - as cited by Yamada and Tsuji, 1980). Absorption rate by rats fed 0.5 or 5 mg of [$^3$H] pyridoxine did not differ (Booth and Brain, 1962). Concurrently, Brain *et al.* (1962 - as cited by Yamada and Tsuji, 1980) concluded that feeding of 1 to 20 mg of pyridoxine to humans lead to an increased excretion of pyridoxine in the urine. The amount excreted was proportional to the dosage fed because only small quantities of the vitamin are stored in the body. These reserves are mainly in the form of pyridoxal-5'-phosphate and pyridoxamine-5'-phosphate. The greatest levels are found in the liver, brain , kidney, spleen, and muscle where it is generally bound to various proteins (i.e., in muscle, vitamin $B_6$ is bound to glycogen phosphorylase) (Henderson, 1984). This binding of the

vitamin to protein may serve to protect it from hydrolysis as well as provide storage of the vitamin.

Pyridoxal, pyridoxamine, and pyridoxine are absorbed rapidly by passive diffusion. Phosphorylated forms must be dephosphorylated by alkaline phosphatase, a membrane-bound enzyme in the intestinal mucosa before absorption (McDowell, 1989). Mucosal cells of the intestine produce pyridoxine kinase, pyridoxine phosphate oxidase, and phosphatases. The presence of these enzymes results in an accumulation of pyridoxal-5'-phosphate by metabolic trapping. However, pyridoxal-5'-phosphate must be once again converted to pyridoxal before it can cross the serosal surface. It is then transported to the liver via the portal circulation.

**Metabolism**

Pyridoxal is converted to pyridoxal-5'-phosphate, which is the most active form of the vitamin, in the liver (McDowell, 1989). Pyridoxal-5'-phosphate is then released into the plasma and is bound to albumin (Dempsey and Christensen, 1962 - as cited by Merrill and Burnham, 1990). This protein-vitamer complex is probably for protection of the active vitamer pyridoxal-5'-phosphate against degradation or metabolism while in the circulatory system (Lumeng and Li, 1980). Plasma contains mostly pyridoxal-5'-phosphate, but there is also a smaller amount of albumin-bound pyridoxal form.

Pyridoxal-5'-phosphate on reaching a target organ must be dephosphorylated by extracellular alkaline phosphatase(s) to pyridoxal which then readily crosses the cell membrane (Brin, 1978). The pyridoxal is then converted back to pyridoxal-5'-phosphate by pyridoxal kinase within the target tissue or cell (Lumeng and Li, 1980). Any excess

pyridoxal-5'-phosphate above the target tissues requirements is oxidized to 4-pyridoxic acid is an inactive end product excreted via the kidneys (Lumeng *et al.*, 1984 - as cited by Bender, 1989).

Pyridoxal-5'-phosphate, pyridoxal, and pyridoxamine-5'-phosphate are involved in a number of metabolic pathways. These include interaction of amino acids, carbohydrates, and fatty acid metabolism and the energy producing citric acid cycle. Over 50 enzymes require vitamin $B_6$ as a cofactor. The coenzyme is generally bound to the enzyme via a Schiff base linkage (Merrill and Burnham, 1990). This linkage occurs between the $C_4$-carboxyaldehyde group of pyridoxal-5'-phosphate and lysine residues in the enzymes protein structure. The specific interaction with lysine serves as the mechanism for initiation of the cofactor action (Morino and Nagashima, 1984 - as cited by Allgood and Cidlowski, 1991).

Vitamin $B_6$ is involved in amino acid and protein metabolism. The role as a cofactor is required by several enzymes including aminotranferases, deaminases, dehydratases, cystathionases, and decarboxylases (Sauberlich, 1985; Snell, 1953 - as cited by McDowell, 1989). Aminotransferase enzymes allow for the removal or addition of amino groups from or to amino acids. Thus, catabolism, synthesis, interconversion, and recycling of amino acids occur. All aminotransferases require pyridoxal-5'-phosphate as a cofactor and nearly all 20 of the amino acids require aminotranferases in order to be metabolized (Guyton, 1991). Aminotranferases and deaminases link amino acids to fatty acid and carbohydrate metabolism (Figure 2) via the formation of pyruvate, acetate, and citric acid cycle intermediates (McDowell, 1989).

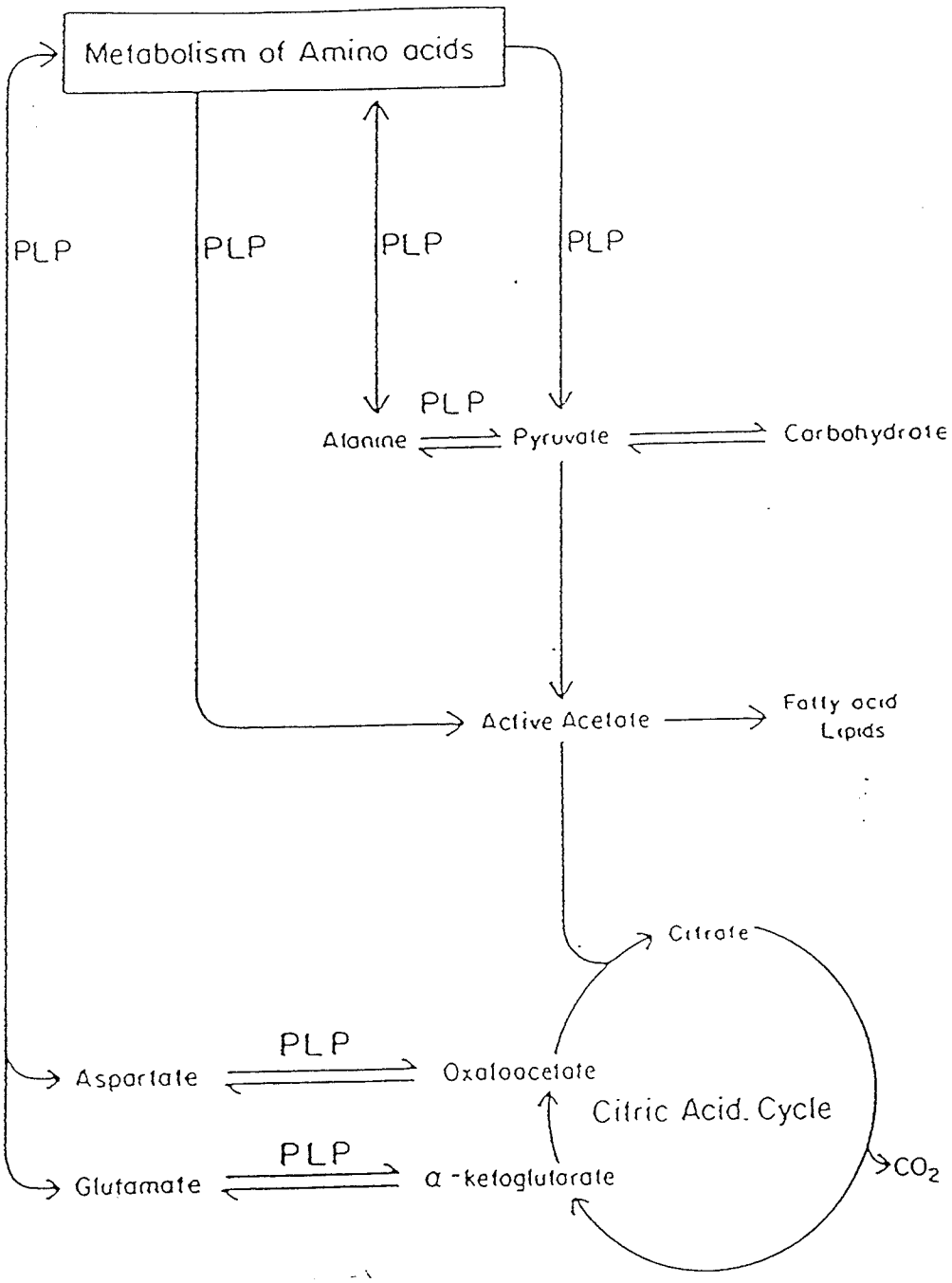



**Figure 2. Pyridoxal-5'-Phosphate (PLP) in Amino Acid, Carbohydrate, and Fatty Acid Metabolism ( Adapted from McDowell, 1989).**

Nonoxidative decarboxylation of amino acids for the synthesis of biogenic amines also requires pyridoxal-5'-phosphate as a coenzyme. These biogenic amines include serotonin, epinephrine, histamine, and taurine which are all involved in homeostasis of the body (neurohormonal action, blood vessel diameter, and essential components of phospholipids and bile acids) (McDowell, 1989)

Vitamin $B_6$ is also important in the conversion of linoleic acid to arachidonic acid. Arachidonic acid is the molecule from which potent biological signalling molecules are produced such as prostaglandins (Lehninger *et al.*, 1993) However, the function of vitamin $B_6$ in this conversion is still controversial (McDowell, 1989). The pyridoxal-5'-phosphate form of vitamin $B_6$ is found to interact with the enzyme glycogen phosphorylase. This enzyme catalyzes the breakdown of glycogen to glucose-1-phosphate, one step in the process of glycogenolysis. However, the vitamer in this case is not a coenzyme but rather serves as a structural component which stabilizes the enzyme's quaternary structure (Sansom *et al.*, 1985). In addition, vitamin $B_6$ is involved in DNA synthesis and cell replication. The vitamin is a cofactor for the enzymes ornithine decarboxylase and S-adenosylmethionine decarboxylase. These enzymes are required for the biosynthesis of certain polyamines which are involved in cell replication (Allgood and Cidlowski, 1991). Furthermore, thymine synthesis is indirectly dependent on pyridoxal-5'-phosphate levels. One of the 4 DNA bases, thymine requires $N_5,N_{10}$-methylene tetrahydrofolic acid (THFA) for it's synthesis. THFA is vitamin $B_6$ dependent ( Allgood and Cidlowski, 1991).

Besides requiring vitamin $B_6$ for metabolism, homeostasis, and cellular

replication, various studies have implicated it's role in the regulation of steroid hormone action which could influence reproduction. Hormones include glucocorticoids, estrogen, progesterone, and androgen. This regulation appears to be at the transcriptional level of gene expression (Allgood and Cidlowski, 1991). Further studies, however, are required. Therefore, vitamin $B_6$ plays a very diverse and important role in mammals, which is still being researched.

## Vitamin $B_6$ and Reproduction

### Blood Levels

Several methods have been proposed for assessing vitamin $B_6$ status in pregnant animals. These methods include the activity of $B_6$ dependent enzymes such as erythrocyte glutamate oxaloacetate aminotransferase (EGOT). A study by Heller *et al.* (1973) found that of 458 pregnant women, 40 to 60% had a suboptimal intake of vitamin $B_6$ to saturate EGOT compared to 300 male and non pregnant female blood donors. EGOT activity was measured at 6 week intervals during gestation, with midgestation having the lowest activity. The results indicate that vitamin $B_6$ supplementation was necessary to maintain normal coenzyme saturation of EGOT. In addition to the testing of enzymes for vitamin $B_6$ status, the tryptophan load test has also been used ( Lumeng *et al.*, 1976; Cleary *et al.*, 1975). This test is based on the principle that vitamin $B_6$ is required for normal tryptophan metabolism (Figure 3). By loading the body with a given amount of tryptophan and measuring the metabolites such as xanthurenic acid excreted

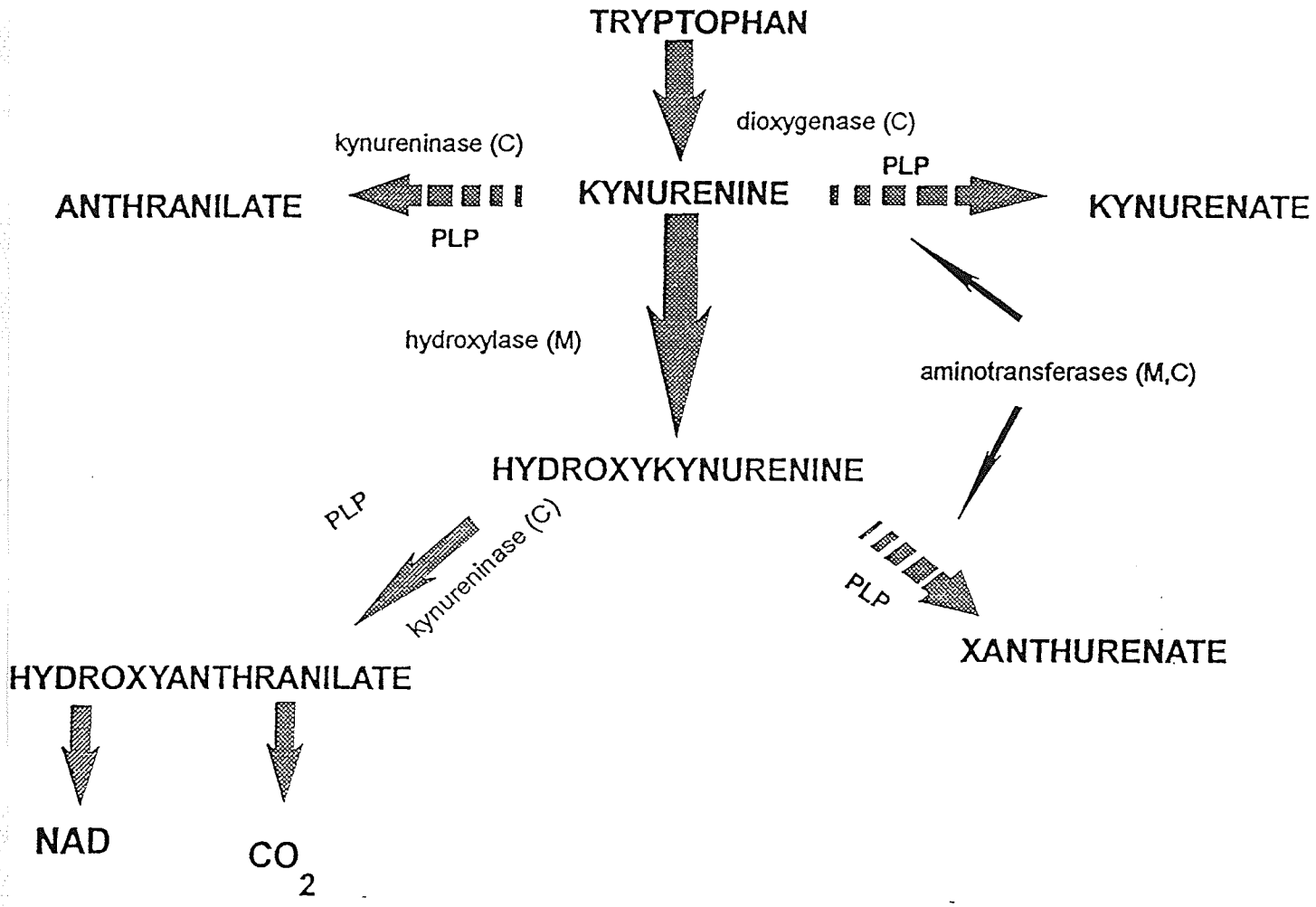



**Figure 3. Initial Steps in the Catabolic Pathway of Tryptophan (Adapted from Shane and Contractor, 1980).**

Under normal physiological conditions the normal metabolic pathway is indicated by solid arrows. The subcellular localization in the cytosol (C) or mitochondrion (M) of pyridoxal-5'-phosphate (PLP) dependent enzymes is indicted.

in the urine, an indication of vitamin $B_6$ status is given. This test, however, is also effected by other factors not related to pregnancy such as bacterial and viral infections, and various hormones (Brown *et al.*, 1987; Bender and Wynick, 1981; Coon and Nagler, 1969 - as cited by Bender, 1989). The most common method used in determining maternal status of vitamin $B_6$ is the level of PLP (pyridoxal-5'-phosphate) in the blood, specifically the plasma.

Several studies have shown that pregnant women have significantly lower plasma PLP levels compared to non pregnant women (Barnard *et al.*, 1987; Cleary *et al.*, 1975; Shane and Contractor, 1975; Hamfelt and Tuvemo, 1972 - as cited by Dempsey, 1978). Supplementation of pregnant women with 2.5 or 4 mg/day of pyridoxine was not found to bring plasma PLP levels up to that of non pregnant women. Supplementation of pregnant women with 10 mg/day of pyridoxine brought both plasma PLP levels and the tryptophan load test close to that of non pregnant women (Lumeng *et al.*, 1976; Cleary *et al.*, 1975). However, a study by Schuster *et al.* (1984) found that an intake of 5.5 and 7.9 mg/day of pyridoxine hydrochloride ( includes diet and supplementation) was required to maintain maternal plasma PLP levels at term. In addition, studies with mice and rats have also found lower plasma PLP levels in pregnant animals compared to non pregnant controls ( Furth-Walker *et al.*, 1989; Slogar and Reynolds, 1980). However, pregnant rats fed a high concentration of vitamin $B_6$ during pregnancy had plasma PLP levels which continued to drop as gestation progressed. Plasma PLP levels were the lowest on the day of delivery (Slogar and Reynolds, 1980).

Several suggestions have been put forth to explain this drop in plasma PLP levels.

One logical explanation is that there is sequestering of the vitamin by the fetus. Studies have shown that the umbilical cord concentration of PLP is 3 to 5 times higher than maternal plasma levels. This suggests that the fetus can accumulate the vitamin at the expense of the mother (Cleary *et al.*, 1975; Contractor and Shane, 1970 - as cited by Shane and Contractor, 1980). Roepke and Kirksey (1979) suggested that this mechanism may ensure adequate reserves for the fetus at birth. The shunting of vitamin $B_6$, however, is not believed to be the sole cause of low plasma PLP during pregnancy as it does not respond well to supplementation (Schuster *et al.*, 1984). Other explanations for this reduction in plasma PLP levels include increased metabolism, increased amino acid turnover, and requirement by $B_6$ dependent enzymes involved in estrogen induction (Brown, 1972).

Other studies have found that although plasma PLP levels decrease during pregnancy, levels in erythrocytes or other forms of the vitamer increase. For example, Furth-Walker *et al.* (1989) found that plasma PLP levels dropped 50% in pregnant mice compared to non pregnant controls, but erythrocyte PLP levels increased 2.9 fold. In addition, although plasma PLP and liver PLP and PMP dropped during pregnancy, brain PLP did not. Therefore, plasma PLP did not give the true vitamin $B_6$ status of the animal. Alkaline phosphatase (ALP), the enzyme which cleaves the phosphate group from PLP, is produced by the placenta. Some researchers believe it may also effect the levels of PLP and PL in the blood (Leibman *et al.*, 1990; McMaster *et al.*, 1964 - as cited by Barnard *et al.*, 1987). Barnard *et al.* (1987) found ALP activity in pregnant women to be 47% higher compared to non pregnant women. They also found that the

total plasma PLP and PL levels (picomoles/ g albumin) were not significantly different between the two groups. From all these studies there is evidence that plasma PLP levels alone are not a good indicator of vitamin $B_6$ status during pregnancy.

Many studies (Shane and Contractor, 1975; Brophy and Siiteri, 1975; Cleary *et al.*, 1975; Hamfelt and Tuvemo, 1972; Brin, 1971; Wachstein *et al.*, 1960 - as cited by Dempsey, 1978) have found that PLP in the plasma drops during pregnancy. Does the drop in plasma PLP indicate a deficiency in vitamin $B_6$ or is it a normal physiological state? Should the plasma PLP levels of pregnant women be brought up to the levels of non pregnant women or merely to the level of average pregnant women? In humans, there have been no real clinical observations of deficiencies even though PLP levels are lower and no data to show increasing PLP levels to that of non pregnant levels will improve the pregnancy or delivery (Dempsey, 1978). Therefore, further studies are required to clarify the status of vitamin $B_6$ in pregnancy.

**Fetal Development**

Vitamin $B_6$ is involved in so many metabolic functions, such as amino acid anabolism and catabolism, carbohydrate metabolism, and heme biosynthesis. Since all the metabolic processes are involved in normal fetal development vitamin $B_6$ will be required for increases in litter size. The role of vitamin $B_6$ in DNA synthesis and the formation of cerebrosides for the central nervous system makes it vital for the fetus (Roepke and Kirksey, 1979). Numerous studies have found that maternal restriction of vitamin $B_6$ in humans caused behavioral abnormalities such as tremors, spontaneous seizures, and irritability in the progeny due to neurochemical changes (McCullough *et*

*al.*, 1990; Coursin, 1955; Synderman *et al.*, 1953 - as cited by Guilarte, 1993). In rats, maternal restriction of vitamin $B_6$ adversely effected a number of morphological parameters within the central nervous system of the progeny. These parameters included development of Purkinge cells dendrites, levels of $\gamma$-aminobutyric acid (GABA), an inhibitory neurotransmitter, myelination of the developing brain, and decreased longevity of neurons in the neocortex (Groziak and Kirksey, 1987; Wasynczuk *et al.*, 1983; Chang *et al.*, 1981; Morre *et al.*, 1978*a* and 1978*b*). The specific mechanism(s) causing the affects on the central nervous system of the developing fetus are still not well understood (Guilarte, 1993).

Roepke and Kirksey (1979) measured vitamin $B_6$ levels in maternal serum at 5 and 7 months gestation and cord serum levels at birth. In addition, they determined Apgar scores for infants at birth. These scores give an indication of infant viability. The Apgar scoring method rates infants on 5 measurements. These include appearance (colour), heart rate, reaction of infant to slapping, effort to breathe, and muscle tone of limbs. The highest score possible is a 10 and the lowest a 0. Generally, the higher the score, the more viable the infant (Apgar, 1974). The study found that infants with $<7$ Apgar score at 1 minute postpartum had mothers with significantly lower maternal serum levels compared to infants with scores of $\geq 7$ taken 1 minute postpartum. Schuster *et al.* (1984) also found that Apgar scores were higher in infants from mothers who were supplemented with 7.5 mg/day or more of vitamin $B_6$.

Although viability scores have been found to be higher in infants from mothers with adequate levels of vitamin $B_6$ in their diets, fetal weights, lengths, and premature

births were not different when vitamin $B_6$ was increased from 0 mg/day to 20 mg/day of supplementation. In addition, maternal parameters such as systolic and diastolic pressure and edema did not differ (Hillman *et al.*, 1963). Kirchgessner *et al.* (1985 ) and Cheney and Beaton (1965) did research with mice and rats and found that increasing vitamin $B_6$ in maternal diets did not increase litter size, fetal weights, number of resorptions, and total litter weight. Moon and Kirksey (1972), however, reported that vitamin $B_6$ deficiency during gestation in rats lead to altered cellular growth and development of organs in progeny during the prenatal period. Rats fed 0.5 mg of vitamin $B_6$ during gestation had progeny with lower DNA content in thymus and kidneys, decreased protein content in the liver, kidneys, and thymus, and decreased RNA content in the liver, heart, and brain. If the deficiency extended into the lactation period, the progeny from these rats did not survive. From these studies, it appears that the effect of vitamin $B_6$ on reproductive parameters is dependent on the status within the mother.

## Vitamin $B_6$ and Nitrogen Retention

As a coenzyme, vitamin $B_6$ in the form of PLP, functions in amino acid metabolism by regulating the activity of several aminotransferases, deaminases, dehydratases, cystathionases, and decarboxylases (Sauberlich, 1985; Snell, 1953 - as cited by McDowell, 1989). In vitro studies have found that the activity of cystathionase, in liver cells, is increased when large amounts of PLP are present (Pascal *et al.*, 1975; Frimpter, 1965 - as cited by Sturman, 1978). Cystathionase is involved in the cleavage of cystathionine to cysteine. It also functions by catalyzing the synthesis of cystathionine

from homoserine and cysteine (Sturman, 1978). Taurine biosynthesis is reduced when vitamin $B_6$ deficiency occurs. This is due to the decrease in cystathionine. The body, however, compensates by decreasing the turnover rate of taurine thereby concentrations remain constant (Sturman, 1973 - as cited by Sturman, 1978). Methionine and cysteine deposition into tissue are generally not affected by vitamin $B_6$ deficiency (Sturman *et al.*, 1970 - as cited by Sturman, 1978). However, during a vitamin $B_6$ deficiency less cystine is incorporated into hair leading to alopecia (Sturman and Cohen, 1971). Addition of cystine to the diet did not alleviate the condition indicating that vitamin $B_6$ is involved more in protein synthesis rather than causing a cysteine deficiency (Sturman, 1978).

Vitamin $B_6$ also plays a major role in tryptophan metabolism (Henderson and Hulse, 1978). Conversion of indole pyruvate to L-tryptophan allows for the utilization of D-tryptophan by some mammalian species (Triebwasser *et al.*, 1976; Langner and Berg, 1955 - as cited by Henderson and Hulse, 1978). Catabolism of tryptophan by PLP dependent tryptophan oxygenase is the first step in the formation of nicotinic acid (Henderson and Hulse, 1978). Nicotinic acid, like vitamin $B_6$, is important for carbohydrate, amino acid and fat metabolism (Maynard *et al.*, 1979).

Only a few studies have been performed to determine the influence of vitamin $B_6$ on nitrogen retention in growing pigs (Wohlbier and Siegel, 1967*b*; Kirchgessner and Friesecke, 1961; Moustgaard *et al.*, 1952 - as cited by ARC, 1981). From these three studies, it was determined that for optimum nitrogen retention, 2.5 mg of vitamin $B_6$ per kg of dietary DM was required. There have been no reported studies of vitamin $B_6$ and nitrogen retention in pregnant sows. From these studies, we could suggest that an

increase in vitamin $B_6$ in the diet of pregnant sows may increase the nitrogen retention in sows, the litter size and number of piglets born alive. Rippel (1967) showed that a daily intake of 230 g of protein with 6,100 Kcal of ME was sufficient to maximize nitrogen retention. Reproductive parameters did not respond to dietary protein levels during the last trimester of gestation. A literature review by Pond (1973) indicated that amount of protein in the diet of gestating sows was not an important factor in determining the litter size or birthweight of piglets. A restriction of protein is believed to affect milk production, therefore, protein is more important in postfarrowing survival of piglets from birth to weaning rather than prefarrowing survival. A more recent study by Dunn and Speer(1988, 1989) indicated that 23.0 g/d of nitrogen intake is sufficient to meet pregnancy requirements provided essential amino acids are fed at the required amount. Therefore, unless vitamin $B_6$ is deficient in the diet and decreases nitrogen utilization there will be no effect on nitrogen retention. If nitrogen retention is increased by supplemental vitamin $B_6$, the increased retention will most likely be beneficial to maternal rather than conceptus tissues.

Plasma urea nitrogen (PUN) is often used to determine the efficiency of nitrogen utilization. A reduction in PUN concentration reflects a decrease in urea synthesis and therefore more efficient utilization of amino acids (Coma *et al.*, 1995). If vitamin $B_6$, through amino acid metabolism, allows for more efficient use of amino acids, then PUN concentration will be reduced and nitrogen retention increased.

## Vitamin $B_6$ and Energy Retention

In addition to amino acid metabolism, PLP also plays a role in carbohydrate and fatty acid metabolism (McDowell. 1989). PLP is a cofactor for the enzyme glycogen phosphorylase. The enzyme converts glycogen to glucose 1-phosphate, the first step in glycogenolysis. Therefore, PLP is involved in the breakdown rather then synthesis of glycogen. This is an important role which allows for adequate glucose levels in the blood. A deficiency of vitamin $B_6$ decreases nitrogen retention as less energy is available for metabolism of amino acids.

In pregnant sows, severe energy intake restriction has been shown to decrease piglet birthweights (Adam and Shearer, 1971; Lodge *et al.*, 1966 - as cited by Pond, 1973). However, the level of energy intake during gestation had little effect on litter size (Gatel *et al.*, 1987; Ponds, 1973). Therefore, if vitamin $B_6$ does improve amino acid utilization and nitrogen retention, which in turn may decrease energy expenditure required for urea synthesis, differences if any, may only be seen for piglet birthweights but not litter size.

## Factors Influencing Vitamin $B_6$ Requirements

### Bioavailability of Vitamin $B_6$ from Feedstuffs

The bioavailability of vitamin $B_6$ from the feed is an important determinator of requirements. That is, feed sources with low bioavailability or low bioactivity of the vitamin may require supplementation. Several factors may affect bioavailability and bioactivity within a diet such as processing, storage, antagonists, and the type of feed.

Food processing such as heating, canning, and freezing have been shown to decrease vitamin $B_6$ bioavailability (Baker, 1995). Yen *et al.* (1976) found that moderate heat (80 to 120°C) of corn increased bioavailability of vitamin $B_6$. However, heat of 160°C decreased availability. In addition to processing losses, storage losses also occur. Storage at room temperature maintained vitamin $B_6$ activity at 76% over a 3 month period. Storage over the same time period, but at a temperature of 37°C decreased the activity to 45% (Adams, 1982 - as cited by Baker, 1995). Gadient (1986 - as cited by Baker, 1995) found that storage of pelleted feeds had an average loss of 20% in vitamin $B_6$ activity over a 3 month period.

Antagonists may interfere with absorption, increase excretion, or interfere with the utilization of the vitamin (Bauerfeind and Miller, 1978). Linatine, a compound found in linseed, has been shown to affect vitamin $B_6$ metabolism rather than absorption (Sauberlich, 1985 - as cited by Baker, 1995). In addition, cooking or heat treating feeds may cause the free aldehyde groups of pyridoxal and pyridoxal-5'-phosphate to react with the amino group of lysine. Pyridoxallysine compounds have been shown to be only 0 to 50% available as a vitamin $B_6$ source (Gregory and Kirk, 1981 - as cited by Baker, 1995). Supplementation of feeds with premixes containing minerals may also affect vitamin $B_6$ bioactivity. Verbeeck (1975) found that minerals in the form of carbonates and oxides decrease the bioactivity of vitamin $B_6$.

The type of feedstuff is an additional factor which effects the bioavailability of vitamin $B_6$. Plants contain the pyridoxine form, while animal products contain pyridoxal and pyridoxal-5'-phosphate. Plant products may also contain pyridoxine glucoside, a

form found in soybeans and sunflower seeds (Kabir *et al.*, 1983). This form appears to have poor utilization in rats (Turembo *et al.*, 1988), but bioavailability of vitamin $B_6$ from this compound is unknown (Baker, 1995). Despite the effects of processing, storage, and antagonists decreasing the bioavailability of vitamin $B_6$ , there is generally sufficient vitamin $B_6$ in the diet to meet most species requirements (Baker, 1995; Easter *et al.*, 1983).

**Animal Health and Age and Vitamin $B_6$ Requirements**

Vitamin $B_6$ is involved in many bodily functions, including immunity. Both humoral and cell-mediated immune response are impaired during vitamin $B_6$ deficiency (Robson et al., 1978). Dobbelstein *et al.* (1974) reported that treating patients deficient in vitamin $B_6$ with oral supplementation corrected the suppression of cell-mediated immunity in uremia. Animals during illness may require additional amounts to maintain immune response, however, no literature can be found to support this idea. Age is also an important factor to consider when determining an animal's requirement. Younger animals will tend to require more vitamin $B_6$ than older animals since increased growth is occurring. The NAS-NRC (1988) recommends 2.0 mg vitamin $B_6$ per kg of feed for 1-5 kg pigs and 1.0 mg per kg of feed for 50-110 kg pigs. However, ARC (1981) recommends 2.5 mg for pigs up to 90 kg.

**Genotype and Vitamin $B_6$ Requirements**

Several studies have investigated the effect of selection of sows for lean growth on reproductive performance (Kuhlers and Jungst, 1992; Cleveland *et al.*, 1988; Fredeen and Mikami, 1986). These studies have found insignificant effects of lean growth

selection on reproductive traits such as litter size or birthweights of piglets. Several studies have shown that mortality from birth to weaning are increased in lean genotype litters (McKay, 1993; Berruecos *et al.*, 1970). This increase in mortality, however, was due more to the increased restlessness of the sow, resulting in a higher incidence of crushing and savaging, rather then being due to nutritional or congenital factors. Lean genotype swine do have increased requirements for amino acids compared to control due to their increased potential for growth. Therefore, increased levels of vitamin $B_6$ may be required to meet this increased metabolism. In addition, unless a vitamin $B_6$ deficiency occurs resulting in a nitrogen imbalance, nitrogen retention in the form of reproductive parameters may not differ between lean genotypes and controls. No studies, however, have looked at feeding vitamin $B_6$ and it's effect on nitrogen retention in pregnant sows.

**Diet Type and Vitamin $B_6$ Requirements**

Morgan *et al.* (1946 - as cited by Miller *et al.*, 1985) found that as protein intake increased, vitamin $B_6$ requirements also increased. High protein diets will also tend to produce abnormal tryptophan metabolism sooner than low protein diets (Miller and Linksweiler, 1967; Baker *et al.*, 1964). However, Miller *et al.* (1985) found that as protein intake increased, the levels of vitamin $B_6$ excreted in the urine decreased. Therefore, more vitamin was retained in the body for catabolism of amino acids. These studies all deal with quantity of protein, but quality of protein has also been tested for an effect on vitamin $B_6$ requirements. A study with rats showed that low quality protein has an adverse effect on vitamin $B_6$ status (Fisher *et al.*, 1984). Sauberlich (1961),

suggested that supplementation of vitamin $B_6$, when certain amino acids are limiting, may allow a larger quantity of these limiting amino acids to be used for protein synthesis and growth instead of catabolized and excreted via the urine.

## Vitamin $B_6$ Requirements for Breeding Swine

Requirements, according to NAS-NRC (1988) and ARC(1981), of vitamin $B_6$ vary depending on the age and the physiological state of the animal. NAS-NRC (1988) recommends 1 mg of vitamin $B_6$/kg of diet for bred gilts, sows, and adult boars. This is based on a corn-soybean meal diet. ARC (1981) estimates that pregnant sows require 1.5 mg of vitamin $B_6$/kg of dietary dry matter. However, they also recognize that requirements may increase under certain conditions.

## Supplementation of Vitamin $B_6$ in Sow Rations

Although many experiments (Schuster *et al.*, 1984; Lumeng *et al.*, 1976; Cleary *et al.*, 1975) have determined the vitamin $B_6$ requirements for pregnant women, very little research has been performed for pregnant sows (Easter *et al.*, 1983; Wohlbier and Siegel, 1967*a* - as cited by ARC, 1981; Ritchie *et al.*, 1960). Ritchie *et al.* (1960) found that supplementation of sow rations from 2 months gestation and continued through lactation with 5 mg of pyridoxine·HCl/kg of diet did not significantly improve reproductive performance such as pigs weaned per litter. Wohlbier and Siegel (1967*a* - as cited by ARC, 1981) with 3 sows per treatment found a nonsignificant increase of 1.3 pigs born/litter when diets fed from service until weaning were supplemented to a total

of 17.2 mg of pyridoxine/kg dietary dry matter. Finally, Easter *et al.* (1983) found that litter size increased not only at birth but remained higher through to weaning when gilts were fed 1.0 ppm of supplemental pyridoxine. Therefore, there seems to be no consistent information on the effect of vitamin $B_6$ supplementation on reproductive performance in sows.

These few studies (Easter *et al.*, 1983; Wohlbier and Siegel, 1967*a* - as cited by ARC, 1981; Ritchie *et al.*, 1960) with pregnant sows and gilts have not compared requirements amongst various breeds. Nor has any studies investigated the requirements of the new lean genotype sows during gestation. There has also been no studies investigating vitamin $B_6$ effect on weaning-to-estrus interval, nitrogen and energy retention during pregnancy.

The objective of this study was to determine the effect of vitamin $B_6$ supplementation on the reproductive performance of lean genotype sows. Nitrogen and energy retention were also examined.

# MATERIALS AND METHODS

## Reproductive Performance Study

### Experimental Diets

A 13% crude protein barley-soybean meal gestation diet was fed to sows from the first day postfarrowing through to the subsequent farrowing (Table 1). Treatment diets differed by the amount of vitamin $B_6$ (in the form of pyridoxine hydrochloride) (Hoffman-LaRoche Ltd., Mississauga, ON) (Table 1). Either, the recommended level of 1 mg (NAS-NRC, 1988) or 15 mg vitamin $B_6$/kg feed was added to the feed at the feed mill prior to pelleting. The feeding of 15 mg was decided upon results of Ritchie *et al.* (1960), and other researchers. Sows were fed 2.0 kg of feed once per day during the gestation period. Water was made available through water nipples at all times. Both diets met or exceeded NAS-NRC (1988) recommended levels of other nutrients for gestating sows (Table 1).

### Experimental Animals

One hundred and eighty one second parity sows of Yorkshire and Hampshire breeds were used in two experiments. Within each breed two genetic lines were used. One line was a control, while the other consisted of animals selected for low backfat thickness over 6 generations. These experiments were of a completely randomized design with a 2 x 2 x 2 factorial arrangement of treatments in which animals from each breed and line were randomly assigned to one of the two test diets (Table 2).

**Table 1. Sow gestation diets, proximate and calculated analysis of diets.**

| Ingredients (%) | Diet 1 | Diet 2 |
|---|---|---|
| Ground barley | 84.00 | 84.00 |
| Soybean meal (44% CP) | 8.50 | 8.50 |
| Animal fat | 3.00 | 3.00 |
| Limestone | 1.25 | 1.25 |
| Monocalcium phosphate (21% P) | 2.50 | 2.50 |
| Iodized salt | 0.35 | 0.35 |
| Vitamin-Trace mineral mix[1] | 0.40 | 0.40 |
| Pyridoxine Hydrochloride[2] (82.7% PN) | 1 mg/kg | 15 mg/kg |
| Total: | 100.00 | 100.00 |

Nutrient Levels (As Fed Basis)

| Parameter | Diet 1 | Diet 2 |
|---|---|---|
| Digestible energy (MJ/kg)[3] | 17.04 | 16.39 |
| Crude protein (%) | 12.85 | 13.58 |
| Crude fibre (%) | 5.55 | 5.84 |
| Crude fat (%)[4] | 4.25 | 4.25 |
| Total calcium (%)[4] | 1.00 | 1.00 |
| Total phosphorus (%)[4] | 0.85 | 0.85 |
| Amino Acid Composition (%) | | |
| Arginine | 0.65 | 0.64 |
| Histidine | 0.28 | 0.29 |
| Isoleucine | 0.31 | 0.32 |
| Leucine | 0.77 | 0.79 |
| Lysine | 0.52 | 0.55 |
| Methionine & Cystine | 0.49 | 0.51 |
| Phenylalanine & Tyrosine | 0.81 | 0.86 |
| Threonine | 0.38 | 0.38 |
| Tryptophan | 0.13 | 0.11 |
| Valine | 0.44 | 0.46 |

[1] The vitamin and mineral premix supplied the following per kg: Vitamin A 10000 IU; Vitamin $D_3$ 600 IU; Vitamin E 50 IU; Vitamin K 4 mg; Vitamin $B_{12}$ 20 mg; Thiamin 2 mg; Riboflavin 5 mg; Niacin 20 mg; Calcium pantothenate 20 mg; Folic acid 5 mg; Biotin 200 $\mu$g; Choline 500 mg; Ethoxyquin 250 mg; Iron 100 mg; Zinc 100 mg; Manganese 30 mg; Copper 10 mg; Iodine 10 mg; Selenium 0.1 mg.

[2] Hoffman-LaRoche Ltd. (Mississauga, ON).

[3] Determined by metabolic studies - $DE = GE_{(feed)} - GE_{(feces)}$.

[4] Calculated analysis.

Table 2. Number of sows in each treatment and number culled from experiment.

| Treatment | Number of Sows | Returns to Estrus | Culled from experiment[1] |
|---|---|---|---|
| H x C x 1 mg | 18 | 4 | 1 |
| H x C x 15 mg | 15 | 8 | 0 |
| H x S x 1 mg | 32 | 12 | 3 |
| H x S x 15 mg | 31 | 9 | 2 |
| Y x C x 1 mg | 21 | 8 | 5 |
| Y x C x 15 mg | 19 | 6 | 2 |
| Y x S x 1 mg | 25 | 7 | 4 |
| Y x S x 15 mg | 20 | 6 | 1 |

[1]Culled for the following reasons: anestrus, leg weakness, death, and abortions.

**Data Collection**

Sows were weighed and ultrasonic backfat measurements taken after weaning, and the date recorded prior to feeding of the test diet. The date, weight, and backfat measurement were also recorded when sows exhibited first estrus. Estrus was determined by the back pressure test and in the presence of a vasectomized boar. A sow was considered in estrus when she stood for both the back pressure test and when she allowed the vasectomized boar to mount. In experiment 1, sows were bred by artificial insemination and breeding took place on the first day of second estrus and about 12 hours later. Breeding date, weight, and backfat probes were recorded. Approximately 18 days postbreeding, sows were observed for signs of returning to estrus. Sows that did not show estrus were excluded from the metabolic study data. Some sows that returned were rebred and used for other projects, and those that did not return were tested at 35 days postbreeding using an ultrasonic machine, Preg-Tone (Renco Corporation, Minneapolis, MN), for confirmation of pregnancy. Sows were housed in gestation crates from weaning until 105 days gestation. Sow backfat measurements and weights were recorded on day 105 and within 24 hours after farrowing.

In experiment 2, sows were weighed at weaning and were bred at first estrus by artificial insemination. Sows were again observed for signs of returning to estrus and were tested for pregnancy using ultrasound at 35 days postbreeding. Sows were housed in individual gestation stalls until day 99 of gestation, when they were weighed and transferred to the farrowing barn, and weighed again within 24 hours of farrowing.

Piglets within each litter were weighed within 24 hours of birth. Number of

stillborn and necrotic piglets were also recorded.

**Statistical Analysis**

The only difference between the two experiments was the time of breeding. Analysis of the data indicates that there was no difference between the experiments for most parameters except weaning-to-estrus weight change in sows and mean piglet birthweights. Therefore, the two experiments were combined and statistically analyzed. Weaning-to-estrus interval, postweaning weight changes, gestational and farrowing weight changes, and backfat of pregnant sows were analyzed by analysis of variance using General Linear Modelling (GLM) in the Statistical Analysis System (SAS Institute Inc., 1988).

The model used was $Y_{ijkl} = \mu+b_i+g_j+d_k+bg_{ij}+bd_{ik}+gd_{jk}+bgd_{ijk}+e_{ijkl}$, where, $Y_{ijkl}$ = the daily weight change of the $l^{th}$ sow on the $k^{th}$ diet in the $j^{th}$ genotype within the $i^{th}$ breed, $\mu$ = the overall daily weight change, $b_i$ = the effect of the $i^{th}$ breed, $g_j$ = the effect of the $j^{th}$ genotype, $d_k$ = the effect of the $k^{th}$ diet, $bg_{ij}$ = the effect of the interaction of the $j^{th}$ genotype and the $i^{th}$ breed, $bd_{ik}$ = the effect of the interaction of the $k^{th}$ diet and the $i^{th}$ breed, $gd_{jk}$ = the effect of the interaction of the $k^{th}$ diet and the $j^{th}$ genotype, $bgd_{ijk}$ = the effect of the interaction of the $k^{th}$ diet, the $j^{th}$ genotype, and the $i^{th}$ breed, $e_{ijkl}$ = random error.

Differences between means were compared by using Bonferonni's multiple comparison test at a significance level of $P<0.05$. In addition, mean litter size, piglets born alive, and birthweights were also determined using GLM analysis. Frequency of

sows that returned to estrus after breeding (not conceived) was determined for each treatment within each experiment and these differences in frequencies between treatments and among stillborn and necrotic piglets were determined using Chi-square ($\chi^2$) analysis ($P<0.05$). Chi-square analysis was calculated according to Ott (1988).

## Metabolic Study

### Experimental Animals

Forty eight second parity pregnant sows were selected at random for metabolism studies. The metabolism studies were performed at approximately 55-60 days gestation in two trials. The metabolic study was performed in two phases. Yorkshire breed sows were tested the first week and Hampshire sows the following week. Sows were allowed to acclimatize to the metabolism crates for 2 days and samples were collected for the next 4 days. Sows were catheterized with Foley Catheters ( Ingram & Bell Scientific, IM-3143-22) for urine collection at this time. Sows were fed 2.0 kg per day of the same diets they received in the reproduction study.

### Data Collection

Sows were weighed at the beginning and the end of the trial and collection was continuous over a 4 day period. Urine was collected in covered pails containing 25 ml of diluted hydrochloric acid (1:3 diluted with water). Feces were collected in plastic covered trays and placed in plastic bags similar to an experiment by Grandhi

and Ibrahim (1990). Volume of urine and weight of feces collected for a 24 hour period were recorded. Representative samples of feces and urine were taken and frozen at -20°C until required for further analysis. Feces and urine samples were later analyzed for dry matter, energy, and nitrogen content.

**Blood Sampling**

Forty-eight second parity sows, from experiment 2, equally representing all treatments, but not necessarily the same sows used in the metabolic study were randomly selected for blood analysis. Blood was collected via the orbital sinus at weaning (basal level), 28 , 55 ,85, and 99 days gestation. Samples were collected in Vacutainer heparinized tubes (Becton Dickinson, Rutherford, NJ) and kept on ice during sampling. Within a half hour of collecting, tubes were centrifuged and plasma pipetted into vials. These vials were then frozen at -20°C until analyzed.

**Analytical Techniques**

Feed samples were collected at random interval during the experiment, mixed and stored. Prior to analysis, samples were mixed and ground in a Tecator cyclotec 1093 sample mill (Hoganas, Sweden). Samples were then dried in a convection oven at 105°C for 24 hours to determine dry matter content. Feces from all 4 days was mixed and a representative sample was freeze-dried in a Virtis Freeze Drier and ground in a Tecator cyclotec 1093 sample mill. Samples were stored in plastic cups

until needed for further analysis. A volume of 50 ml of urine was filtered and mixed with 2 g of cellufil. The samples were then freeze-dried in a Virtis Freeze Drier at -50°C for 72 hours. Dried samples were then ground using a mortar and pestle, placed in plastic capped cups and stored in a dessicator until needed for further analysis. Urine dry matter content was determined by weighing the dried mixture and correcting for the cellufil blank sample.

Dry matter, nitrogen and fat in feed and feces samples were determined according to methods described by the Association of Official Analytical Chemists (1990). Gross energy was determined by using an adiabatic oxygen bomb calorimeter (Parr, model 1241). Nitrogen content of urine samples were determined using a similar method as that used for feces and feed samples except 1 ml of urine was used for analysis. Energy content of urine was determined using the same method used for feces and feed samples except gross energy was corrected for the cellufil blank. Fat was determined using ether extraction according to the method described by the Association of Official Analytical Chemists (1990).

Plasma samples were analyzed for urea nitrogen concentrations using a standard kit (Procedure No. 535) from Sigma Diagnostics (St. Louis, MO).

**Calculations Used in Determining Nutrient Intakes, Digestibilities, and Retentions**

Dry matter intake (g) = Amount feed (g) x dry matter content of feed (%)

Fat intake (g) = Fat in feed (%) x dry matter intake (g)

Energy intake (MJ)=((Energy in feed (Kcal/g)x dry matter intake (g)) x 4.185)/1000)

Nitrogen intake (g) = Nitrogen in feed (%) x dry matter intake (g)

Dry matter digestibility (%) = ((DM intake - fecal DM)/ DM intake) x 100

Fat digestibility (%) = ((fat intake - fecal fat)/ fat intake) x 100

Energy digestibility (%) = ((energy intake - fecal energy)/ energy intake) x 100

Nitrogen digestibility (%) = ((nitrogen intake - fecal nitrogen)/ nitrogen intake) x 100

Dry matter retention (%) = ((DM intake - fecal DM - urine DM)/ DM intake) x 100

Energy retention (%) = (( energy intake - fecal energy - urine energy)/ energy intake) x 100

Nitrogen retention (%) = ((nitrogen intake - fecal nitrogen - urine nitrogen)/ nitrogen intake) x 100

Energy retention does not include heat loss as sows were housed in an controlled environment.

**Statistical Analysis**

Analysis of the data indicates that there was no difference between the experiments for most parameters except nutrient intakes. Therefore, the two experiments were combined and statistically analyzed. Dry matter, energy, and nitrogen intakes, digestibilities, and retentions and fat intake and digestibility were determined by analysis of variance using General Linear Modelling (GLM) in the Statistical Analysis System (SAS Institute Inc., 1988). Differences between treatments were determined using Bonferroni's multiple comparison test ($P > 0.05$).

Blood samples were divided into bleeding periods and plasma urea nitrogen levels were determined by analysis of variance using GLM. Treatment differences were determined using Bonferroni's multiple comparison test ($P<0.05$).

## RESULTS AND DISCUSSION

### Weaning-to Estrus Interval and Postweaning, Gestational, and Farrowing Weight Changes

An increase in supplemental vitamin $B_6$ in the diet significantly ($P<0.05$) decreased the weaning-to-estrus interval (Table 3). Vitamin $B_6$ supplemented in sow rations at 15 mg/kg of feed reduced the weaning-to-estrus interval by 1 day compared to sows fed 1 mg of vitamin $B_6$. There were no significant differences between breeds or genotypes, nor were any interactions between breeds, genetic lines, or diets observed. No studies have investigated the influence of supplemental vitamin $B_6$ on weaning-to-estrus interval. Ritchie *et al.* (1960) only reported the effect of supplemental vitamin $B_6$ from 2 months gestation through lactation, while Wohlbier and Siegel (1967*a* - as cited by ARC, 1981) fed supplemental vitamin $B_6$ from breeding through gestation. Although there are no studies showing that supplemental vitamin $B_6$ influences weaning-to-estrus interval, perhaps the vitamin's role in amino acid and energy metabolism and

Table 3. Dietary vitamin $B_6$ effect on weaning-to-estrus interval and weight changes in second parity sows.

| | Variables | | | |
|---|---|---|---|---|
| Factors: | Weaning-to-estrus (days) | Weaning wt. change (kg)[c] | Estrus wt. change (kg)[d] | Gestational wt. gain (kg)[e] |
| Diet (D) | * | ns | ns | ns |
| 1 mg/kg | 5.45 | -13.46 | -0.31 | 34.47 |
| 15 mg/kg | 4.45 | -14.09 | -0.18 | 34.29 |
| SE[a] | 0.32 | 0.79 | 0.44 | 1.43 |
| Genotype (G) | ns | ns | ns | * |
| Control (C) | 5.17 | -12.93 | 0.24 | 37.40 |
| Lean (L) | 4.73 | -14.62 | -0.73 | 31.35 |
| SE[a] | 0.32 | 0.79 | 0.43 | 1.42 |
| Breed (B) | ns | * | ns | ns |
| Hampshire (H) | 5.08 | -15.22 | -0.20 | 36.09 |
| Yorkshire (Y) | 4.82 | -12.33 | -0.29 | 32.66 |
| SE[a] | 0.32 | 0.79 | 0.44 | 1.43 |
| B x G | ns | ns | ns | * |
| H x C | 5.36 | -14.19 | 0.56 | 37.10 |
| H x L | 4.80 | -16.25 | -0.96 | 35.08 |
| Y x C | 4.98 | -11.66 | -0.09 | 37.70 |
| Y x L | 4.67 | -13.00 | -0.50 | 27.63 |
| SE[a] | 0.45 | 1.11 | 0.61 | 1.98 |
| B x D[b] | ns | ns | ns | ns |
| G x D[b] | ns | ns | ns | ns |
| B x G x D[b] | ns | ns | ns | ns |

*=P<0.05 ns=non significant, P>0.05.

[a] SE refers to standard error of Least square means.

[b] means are not presented for these sets of non significant effects.

[c] weaning to first estrus weight change.

[d] first estrus to breeding weight change in experiment 1 only.

[e] breeding to farrowing weight change.

steroid hormone function may explain this slight decrease in the weaning-to-estrus interval in sows fed 15 mg of vitamin $B_6$.

Increased supplementation of vitamin $B_6$ had no significant effect on postweaning or gestational weight changes (Table 3). Hampshire sows lost significantly ($P<0.05$) more weight in the weaning-to-first estrus period compared to Yorkshire sows, but weight changes from first estrus to breeding and during gestation did not significantly differ between the two breeds. The genotype did not significantly affect the weight changes during the postweaning period. Lean sows, however, gained significantly ($P<0.05$) less weight during gestation compared to control sows. A significant ($P<0.05$) breed x line interaction was observed for gestational weight change. This interaction indicates that lean Yorkshire sows gained less weight during gestation compared to lean Hampshire sows. The difference is probably due to differing genetic potentials of Yorkshire and Hampshire sows, as Yorkshire sows tend to be leaner (Grandhi, personal communication).

## Backfat Measurements from Weaning through to Farrowing

The level of vitamin $B_6$ in the diet did not significantly ($P>0.05$) affect the backfat measurements of sows from weaning through to farrowing (Table 4). However, backfat loss from weaning through breeding was less in sows fed 15 mg of vitamin $B_6$ compared to sows fed only 1 mg. Lean genotype sows had significantly ($P<0.01$)

Table 4. Dietary vitamin $B_6$ effect on backfat changes in second parity sows.

| Factors: | Variables | | | |
|---|---|---|---|---|
| | Weaning backfat (mm) | First estrus backfat (mm) | Breeding backfat (mm) | Farrowing backfat (mm) |
| Diet (D) | ns | ns | ns | ns |
| 1 mg/kg | 16.16 | 13.51 | 14.01 | 14.25 |
| 15 mg/kg | 16.11 | 14.79 | 15.27 | 15.38 |
| SE[a] | 0.51 | 0.63 | 0.60 | 0.77 |
| Genotype (G) | ** | ** | * | * |
| Control (C) | 17.48 | 15.42 | 15.71 | 17.20 |
| Lean (L) | 14.79 | 12.89 | 13.57 | 12.42 |
| SE[a] | 0.50 | 0.62 | 0.60 | 0.76 |
| Breed (B) | ** | ns | ns | ns |
| Hampshire (H) | 17.12 | 14.64 | 15.04 | 15.41 |
| Yorkshire (Y) | 15.15 | 13.67 | 14.23 | 14.21 |
| SE[a] | 0.51 | 0.63 | 0.60 | 0.77 |
| Breed x Genotype | ns | * | * | ns |
| H x C | 19.09 | 16.43 | 17.10 | 17.53 |
| H x L | 15.14 | 12.31 | 12.99 | 13.29 |
| Y x C | 15.86 | 13.88 | 14.31 | 16.88 |
| Y x L | 14.44 | 13.46 | 14.15 | 11.55 |
| SE[a] | 0.94 | 0.88 | 0.84 | 1.07 |
| Breed x Diet[b] | ns | ns | ns | ns |
| Genotype x Diet[b] | ns | ns | ns | ns |
| B x G x D[b] | ns | ns | ns | ns |

*=P<0.05 **=P<0.01 ns=non significant, P>0.05.

[a] SE refers to standard error of Least square means.

[b] means are not presented for these sets of non significant effects.

lower weaning and first estrus backfats compared to controls. Backfat at breeding and farrowing was also significantly ($P<0.05$) lower in lean genotype sows. Perhaps the extra weight gain in control sows was due to the extra deposition of fat. Hampshire sows had significantly ($P<0.01$) higher backfat measurements compared to Yorkshire sows at weaning. All other backfat measurements did not differ significantly between breeds.

There was a breed x genotype interaction for backfat at first estrus and breeding. A greater difference was observed between Hampshire x control sows and Hampshire x lean sows compared to Yorkshire x control sows and Yorkshire x lean sows. Since Yorkshire sows are known to be leaner than Hampshire sows perhaps the genetic potential of lean Yorkshire sows is very similar to control Yorkshire sows. In this present study, lean Hampshire sows appear to be genetically quite different from control Hampshire sows (Grandhi, unpublished data).

## Conception Rate, Stillbirths, and Necrotic Piglets

There were no significant ($P>0.05$) differences between breeds, genetic lines, or level of vitamin $B_6$ in the diet on conception rates (Table 5). An interesting observation of this study is that Hampshire sows had significantly ($P<0.05$) greater weight loss from weaning-to first estrus but had similar conception rates as Yorkshire sows. This loss in body weight was not large enough to affect reproduction, as

Table 5. Dietary vitamin $B_6$ effects on the conception rate in second parity sows.

| | | Proportions | | |
|---|---|---|---|---|
| | Number of Sows | Pregnant | Non pregnant | $\chi^2$ |
| Diet (D) | | | | 0[a] ns |
| 1 mg/kg | 82 | .61 | .39 | |
| 15 mg/kg | 77 | .62 | .38 | |
| Genotype (G) | | | | .01[a] ns |
| Control (C) | 65 | .58 | .42 | |
| Lean (L) | 94 | .64 | .36 | |
| Breed (B) | | | | 0 [a] ns |
| Hampshire (H) | 90 | .62 | .38 | |
| Yorkshire (Y) | 69 | .61 | .39 | |

[a] for a $\chi^2$ test that proportions of pregnant and non-pregnant are the same for the two groups.

backfat measurements at estrus did not differ between the two breeds. There were no significant differences between breeds, genotypes, or diets on stillbirths or necrotic piglets (Table 6).

**Litter size and Mean Birthweight of Piglets**

Sows fed 15 mg of vitamin $B_6$ tended (P=0.09) to have increased litter sizes of 0.7 piglets compared to sows fed 1 mg of vitamin $B_6$ (Table 7). This is similar to the results of an experiment by Wohlbier and Siegel (1967*a* - as cited by ARC, 1981) who found a non significant increase of 1.3 piglets per litter when sows were supplemented with vitamin $B_6$. Another interesting observation is that Yorkshire x lean x 15 mg of vitamin $B_6$ treatment sows had an increase in littersize of 1.75 piglets compared to Yorkshire x lean x 1 mg of vitamin $B_6$ treatment sows. This indicates that vitamin $B_6$ improves either ovulation rates or embryo survival rates in lean Yorkshire sows. Control Hampshire sows fed 15 mg of vitamin $B_6$ had larger litters compared to control Hampshire sows fed 1 mg. Hampshire sows tend to have higher backfat measurements compared to Yorkshire sows. However, a review by Aherne and Kirkwood (1985) indicated that over conditioned (ie. fat) sows may have reduced conception rates and poor embryo survival due to low levels of progesterone mediated by the action of mixed function oxidase enzyme in the liver. Since vitamin $B_6$ is involved in the regulation of steroid hormones such as progesterone, perhaps despite higher backfats in control Hampshire sows, embryo survival was not affected due to

Table 6. Dietary vitamin $B_6$ effect on the proportion of stillbirths, necrotics, and livebirths in second parity sows.

| | | Proportions | | | |
|---|---|---|---|---|---|
| | Number of Piglets | Stillborn | Necrotics | Born Alive | $\chi^2$ |
| Diet (D)[b] | | | | | 3.04[a] ns |
| 1 mg/kg | 544 | .06 | .01 | .93 | |
| 15 mg/kg | 547 | .07 | .02 | .91 | |
| Genotype (G)[b] | | | | | .01[a] ns |
| Control (C) | 412 | .05 | .02 | .93 | |
| Lean (L) | 679 | .07 | .02 | .91 | |
| Breed (B)[b] | | | | | 1.39[a] ns |
| Hampshire (H) | 645 | .06 | .03 | .91 | |
| Yorkshire (Y) | 446 | .07 | 0 | .93 | |

[a] for a $\chi^2$ test that proportions of pregnant and non-pregnant are the same for the two groups.

[b] number of sows (n) per treatment:

| | | |
|---|---|---|
| diet | 1 | n=82 |
| | 15 | n=77 |
| genotype | C | n=65 |
| | L | n=94 |
| breed | H | n=90 |
| | Y | n=69 |

Table 7. Dietary vitamin $B_6$ effect on littersize and mean birthweights of piglets in second parity sows.

| | Variables | |
|---|---|---|
| Factors: | Litter Size | Mean Birthweight(kg) |
| Diet (D) | ns | ns |
| 1 mg/kg | 10.81 | 1.47 |
| 15 mg/kg | 11.54 | 1.40 |
| SE[a] | 0.30 | 0.03 |
| Genotype (G) | ns | ns |
| Control (C) | 11.32 | 1.44 |
| Lean (L) | 11.03 | 1.43 |
| SE[a] | 0.30 | 0.03 |
| Breed (B) | ns | ns |
| Hampshire (H) | 11.57 | 1.40 |
| Yorkshire (Y) | 10.78 | 1.47 |
| SE[a] | 0.30 | 0.03 |
| Breed x Genotype[b] | ns | ns |
| Breed x Diet[b] | ns | ns |
| Genotype x Diet[b] | ns | ns |
| Breed x Genotype x Diet | * | ns |
| H x C x 1 | 10.92 | 1.46 |
| H x C x 15 | 12.50 | 1.31 |
| H x L x 1 | 11.71 | 1.41 |
| H x L x 15 | 11.17 | 1.43 |
| Y x C x 1 | 10.88 | 1.54 |
| Y x C x 15 | 11.00 | 1.46 |
| Y x L x 1 | 9.75 | 1.48 |
| Y x L x 15 | 11.50 | 1.39 |
| SE[a] | 0.59 | 0.07 |

*=$P<0.05$ ns=non significant, $P>0.05$.

[a]SE refers to standard error of Least square means.

[b] means are not presented for these sets of non significant effects.

influence of vitamin $B_6$. There were no significant ($P>0.05$) differences between breeds, genotypes, or level of vitamin $B_6$ in the diet on littersize.

Neither breed, genetic line, nor level of vitamin $B_6$ had any significant ($P>0.05$) effect on the mean birthweight of piglets (Table 7). There were no interactions among breed, genetic line, or diet on the mean birthweight of piglets. A previous study by Ritchie *et al.* (1960) found no significant differences in litter size or birthweights of piglets when sows were supplemented with vitamin $B_6$. Yorkshire x lean sows fed 15 mg of vitamin $B_6$ tended to have larger litter sizes ($P=0.09$) but piglets tended to weigh less at birth ($P=0.13$) compared to sows fed 1 mg. Hampshire sows had the same tendency, with larger litter sizes ($P= 0.06$) and smaller birthweights ($P=0.17$). Limited uterine capacity may be the limiting factor causing these smaller birthweights (DeRoth and Downie, 1976). Grandhi (unpublished data) observed that second parity Yorkshire sows tended to produce larger litters and higher birthweights compared to Hampshire sows. However, in this study, Yorkshire sows tended to out perform Hampshire sows in litter size and backfats, but not piglet birthweights.

## Nutrient Retention During A Metabolism Study

The level of vitamin $B_6$ in the diet significantly ($P<0.05$) affected the amount of dry matter retained (Table 8). Sows receiving 15 mg of vitamin $B_6$ had significantly ($P<0.05$) lower dry matter retention compared to sows receiving 1 mg. Dry matter

Table 8. Dietary vitamin $B_6$ effect on nutrient retention in second parity sows during a metabolism study.

| | Variables | | |
|---|---|---|---|
| Factors: | Dry matter retention (%) | Energy retention (%) | Nitrogen retention (%) |
| Diet (D) | * | ns | ns |
| 1 mg/kg | 76.17 | 77.79 | 16.53 |
| 15 mg/kg | 74.24 | 75.99 | 21.97 |
| SE[a] | 0.58 | 0.68 | 2.69 |
| Genotype (G) | ns | ns | ns |
| Control (C) | 74.86 | 76.58 | 19.12 |
| Lean (L) | 75.55 | 77.21 | 19.37 |
| SE[a] | 0.58 | 0.68 | 2.70 |
| Breed (B) | * | * | ** |
| Hampshire (H) | 76.28 | 78.04 | 30.44 |
| Yorkshire (Y) | 74.13 | 75.77 | 8.06 |
| SE[a] | 0.60 | 0.70 | 2.76 |
| Breed x Genotype[b] | ns | ns | ns |
| Breed x Diet[b] | ns | ns | ns |
| Genotype x Diet[b] | ns | ns | ns |
| B x G x D[b] | ns | ns | ns |

*=P<0.05 **=P<0.01 ns=non significant, P>0.05.

[a] SE refers to standard error of Least square means.

[b] means are not presented for these sets of non significant effects.

intake (Table 9) and digestibility (Table 10) were also found to be significantly ($P<0.01$) different between the two diets. Sows fed 15 mg had lower dry matter intakes and digestibilities compared to sows fed 1 mg. Since the diets did not differ except for the level of vitamin $B_6$, intake differences are due to artifacts of analysis. These observations explain the lower dry matter retention in sows fed 15 mg. There were no significant differences between genotypes for dry matter intake, digestibility, or retention. Hampshire sows retained significantly ($P<0.05$) more dry matter compared to Yorkshire sows. However, dry matter intakes and digestibilities did not differ between the two breeds. No breed, genotype, or diet interactions were observed for dry matter intake, digestibility, or retention.

Energy retention was not significantly affected by the level of vitamin $B_6$ in the diet (Table 8). However, sows fed 15 mg of vitamin $B_6$ tended to have slightly lower energy retention compared to sows fed 1 mg. Energy intake (Table 9) did not differ between the two diets, but energy digestibility (Table 10) was significantly ($P<0.01$) lower in sows fed 15 mg of vitamin $B_6$. There were no significant differences between genotypes for energy intake, digestibility, or retention. Hampshire sows had significantly ($P<0.05$) higher energy retention compared to Yorkshire sows. However, energy intakes and digestibility did not differ between the two breeds. No breed, genotype, or diet interactions were observed for energy intake, digestibility, or retention. Fat intakes (Table 9) and digestibility (Table 10) did not differ between genotypes or breeds. However, sows fed 15 mg of vitamin $B_6$ had significantly ($P<0.01$) higher fat intakes compared to sows fed 1 mg. This was due to the dry matter content of the diets

Table 9. Dietary vitamin $B_6$ effect on the daily nutrient intakes of second parity sow during a metabolism study.

| | Variables | | | |
|---|---|---|---|---|
| Factors: | Dry matter intake (g) | Fat intake (g) | Energy intake (MJ) | Nitrogen intake (g) |
| Diet (D) | ** | ** | ns | ** |
| 1 mg/kg | 1795.49 | 76.39 | 34.74 | 45.31 |
| 15 mg/kg | 1777.00 | 75.50 | 34.39 | 47.74 |
| SE[a] | 0.70 | 0.12 | 0.64 | 0.63 |
| Genotype (G) | ns | ns | ns | ns |
| Control (C) | 1787.41 | 75.96 | 34.51 | 46.57 |
| Lean (L) | 1786.94 | 75.94 | 34.61 | 46.48 |
| SE[a] | 3.01 | 0.13 | 0.64 | 0.63 |
| Breed (B) | ns | ns | ns | ns |
| Hampshire (H) | 1785.57 | 76.02 | 34.22 | 46.88 |
| Yorkshire (Y) | 1788.78 | 75.88 | 34.91 | 45.17 |
| SE[a] | 3.09 | 0.13 | 0.66 | 0.65 |
| Breed x Genotype[b] | ns | ns | ns | ns |
| Breed x Diet[b] | ns | ns | ns | ns |
| Genotype x Diet[b] | ns | ns | ns | ns |
| B x G x D[b] | ns | ns | ns | ns |

**=P<0.01 ns=non significant, P>0.05.

[a] SE refers to standard error of Least square means.

[b] means are not presented for these sets of non significant effects.

Table 10. Dietary vitamin $B_6$ effect on the apparent nutrient digestibility in second parity sows during a metabolism study.

| | Variables | | | |
|---|---|---|---|---|
| Factors: | Dry matter digestibility (%) | Fat digestibility (%) | Energy digestibility (%) | Nitrogen digestibility (%) |
| Diet (D) | ** | ns | ** | ns |
| 1 mg/kg | 83.69 | 74.51 | 86.37 | 86.25 |
| 15 mg/kg | 81.77 | 70.23 | 84.70 | 86.18 |
| SE[a] | 0.45 | 1.58 | 0.40 | 0.37 |
| Genotype (G) | ns | ns | ns | ns |
| Control (C) | 82.54 | 73.04 | 85.40 | 85.94 |
| Lean (L) | 82.93 | 71.74 | 85.67 | 86.48 |
| SE[a] | 0.45 | 1.59 | 0.40 | 0.37 |
| Breed (B) | ns | ns | ns | ns |
| Hampshire (H) | 82.73 | 72.68 | 85.41 | 86.09 |
| Yorkshire (Y) | 82.73 | 72.10 | 85.66 | 86.34 |
| SE[a] | 0.47 | 1.63 | 0.41 | 0.38 |
| Breed x Genotype[b] | ns | ns | ns | ns |
| Breed x Diet[b] | ns | ns | ns | ns |
| Genotype x Diet[b] | ns | ns | ns | ns |
| B x G x D[b] | ns | ns | ns | ns |

**=$P<0.01$ ns=non significant, $P>0.05$.

[a] SE refers to standard error of Least square means.

[b] means are not presented for these sets of non significant effects.

(artifacts of analysis). Fat digestibility did not differ between the two diets and no interactions were observed between breeds, genotypes, or diets for fat intake or digestibility.

Nitrogen retention did not differ significantly between diets (Table 8). Sows fed 15 mg of vitamin $B_6$ tended ($P=0.16$) to have higher nitrogen retention compared to sows fed 1 mg ( 21.97% versus 16.53%). The 5% increase in nitrogen retention in sows fed 15 mg of vitamin $B_6$ was not significant due to the large standard error. Nitrogen intakes were also significantly ($P<0.01$) higher in sows fed 15 mg of vitamin $B_6$ (Table 9), however, nitrogen digestibility did not differ between the two diets (Table 10). This increased nitrogen intake does not explain the improved nitrogen retention in sows fed 15 mg of vitamin $B_6$. Evert and Dekker (1994) observed that as nitrogen intake increases, nitrogen excretion also increases and nitrogen retention does not differ. There were no significant differences in nitrogen intake, digestibility, or retention between the two genotypes.

Nitrogen intake and digestibility did not differ between Hampshire and Yorkshire sows. However, Hampshire sows had significantly ($P<0.01$) greater nitrogen retention compared to Yorkshire sows. No interactions were observed between breeds, genotypes, or diets.

During gestation a greater efficiency of protein utilization has been reported (Heap and Lodge, 1967; Salmon-Legagneur, 1965; Rombauts, 1962 - as cited by ARC, 1981). Elsley et al. (1966) found a 9% increase in nitrogen retention in pregnant gilts compared to non pregnant gilts fed identical levels of protein over a 110 day period. The study

also found that as gestation progressed, the mean nitrogen retention increased. Everts and Dekker (1994) found that second parity sows fed 42.1 g/day of nitrogen retained 15.7 g of nitrogen during mid gestation. However, the results of this study show that nitrogen retention is quite low in comparison. Energy restriction may be the cause of lower nitrogen retention seen in the present experiment.

A study by Jones and Maxwell (1982) found that mid gestation gilts retained 17.8 g of nitrogen per day during a summer trial and 4.1 g/day in a winter trial. In the current experiment, nitrogen retention was comparable to the winter study of Jones and Maxwell (1982). However, the sows in this experiment were housed in an environmentally controlled atmosphere which does not explain the low retentions. Sows in the current experiment were restricted fed which may account for the poor nitrogen retentions observed. If energy intake was not adequate to meet the animal's requirements, protein would be catabolized and the carbon skeletons utilized as energy.

## Plasma Urea Nitrogen Concentrations During the Postweaning and Gestational Periods

The concentration of plasma urea nitrogen (PUN) from weaning through breeding and gestation did not differ significantly between the two diets (Table 11). If vitamin $B_6$ were to improve amino acid utilization a drop in PUN concentration would have occurred. No significant differences were seen in PUN levels for lean genotype sows. It is expected that these lean genotype sows would have lower PUN levels as they

Table 11. Dietary vitamin $B_6$ effect on the plasma urea nitrogen concentration (mg/dL) in second parity sows.

| | Variables | | | | |
|---|---|---|---|---|---|
| Factors: | Basal[c] | 28 Days Gestation | 55 Days Gestation | 85 Days Gestation | 99 Days Gestation |
| Diet (D) | ns | ns | ns | ns | ns |
| 1 mg/kg | 18.59 | 18.26 | 19.25 | 18.67 | 19.28 |
| 15 mg/kg | 18.92 | 18.88 | 18.09 | 17.63 | 17.73 |
| SE[a] | 1.15 | 1.13 | 0.77 | 0.82 | 0.88 |
| Genotype (G) | ns | ns | ns | ns | ns |
| Control (C) | 18.81 | 19.44 | 18.63 | 18.87 | 18.50 |
| Lean (L) | 18.69 | 17.69 | 18.71 | 17.43 | 18.51 |
| SE[a] | 1.15 | 1.12 | 0.77 | 0.82 | 0.88 |
| Breed (B) | ns | ns | ** | ** | ** |
| Hampshire (H) | 18.41 | 18.33 | 17.10 | 16.29 | 16.94 |
| Yorkshire (Y) | 19.10 | 18.80 | 20.25 | 20.01 | 20.07 |
| SE[a] | 1.15 | 1.13 | 0.77 | 0.82 | 0.88 |
| Breed x Genotype | ns | ns | ns | ns | ** |
| H x C | 16.92 | 19.10 | 16.96 | 16.83 | 15.01 |
| H x L | 19.90 | 17.57 | 17.24 | 15.75 | 18.87 |
| Y x C | 20.71 | 19.78 | 20.31 | 20.91 | 21.99 |
| Y x L | 17.49 | 17.82 | 20.18 | 19.11 | 18.16 |
| SE[a] | 1.63 | 1.59 | 1.04 | 1.16 | 1.24 |
| Breed x Diet[b] | ns | ns | ns | ns | ns |
| Genotype x Diet[b] | ns | ns | ns | ns | ns |
| B x G x D[b] | ns | ns | ns | ns | ns |

**=P<0.01 ns=non significant, P>0.05.

[a] SE refers to standard error of Least square means.

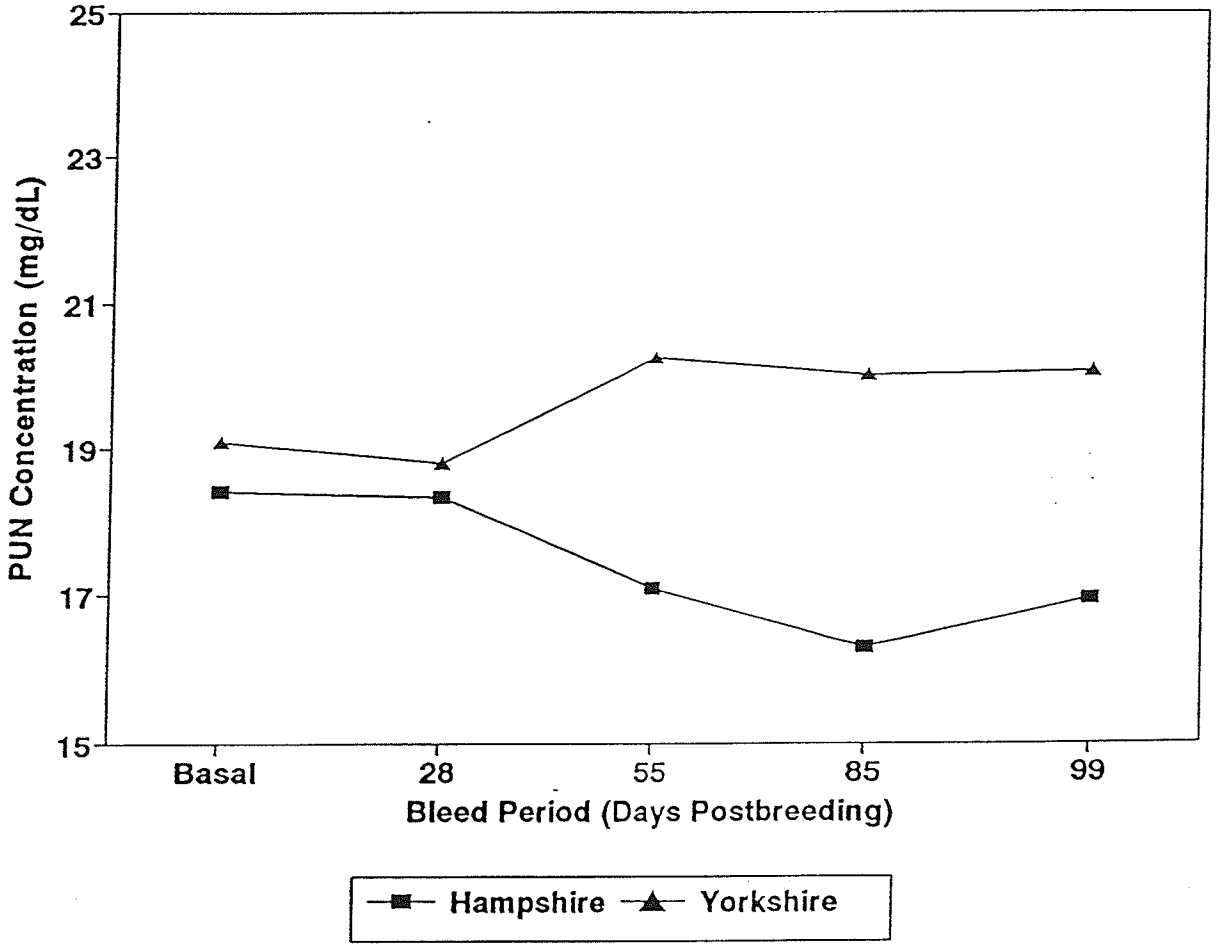
[b] means are not presented for these sets of non significant effects.

[c] Basal level taken at weaning prior to feeding experimental diets.

utilize protein and energy better than other nonlean genotypes. Hampshire sows had slightly lower PUN concentrations at weaning and 28 days gestation compared to Yorkshire

sows. However, from 55 to 99 days gestation Hampshire sows had significantly ($P<0.01$) lower PUN levels compared to Yorkshire sows. This can be visualized by Figure 4. The lower PUN levels in Hampshire sows may explain the significantly ($P<0.01$) higher nitrogen retention in Hampshire sows versus Yorkshire sows (30.44 % versus 8.06 %) (Table 8). On day 99 of gestation it was observed that Hampshire control sows had lower PUN concentrations compared to Hampshire lean and Yorkshire control and lean genotypes. As explained early, perhaps control Yorkshire sows are similar genetically to lean Yorkshire sows while control and lean Hampshire sows are genetically very different. If Yorkshire sows are not receiving adequate energy to meet their requirements, PUN levels are increased due to increased catabolism of protein. A study by Sticker *et al.* (1995) found that energy restriction of mares lead to significant ($P=0.013$) increases in PUN levels. Perhaps, Hampshire sows may not require the same amount of energy as they mature earlier and have less lean tissue mass.

Figure 4. Plasma Urea Nitrogen Concentrations in Hampshire and Yorkshire Second Parity Sows From Weaning through Gestation.


25
23
21
19
17
15
PUN Concentration (mg/dL)
Basal
28
55
85
99
Bleed Period (Days Postbreeding)
Hampshire
Yorkshire

# GENERAL DISCUSSION

The current study determined the effect of feeding supplemental vitamin $B_6$ to sows during the postweaning and gestational periods. Diets containing either 1 mg (NAS-NRC (1988) recommendation) or 15 mg of vitamin $B_6$ were fed. Reproductive parameters and a metabolic study were used to determine whether supplemental levels of vitamin $B_6$ in sow diets are necessary to improve sow performance.

Increasing the level of vitamin $B_6$ in the diet of sows does not improve all areas of reproduction. Improvements in weaning-to-estrus interval ($P<0.05$) and litter size ($P<0.10$) were observed. However, conception rates, birthweights, and piglets born alive were unaffected by vitamin $B_6$ levels. In addition to improved weaning-to-estrus interval, litter size was also slightly increased in sows fed 15 mg of vitamin $B_6$. The metabolic study revealed that the level of vitamin $B_6$ in the diet did not significantly improve nutrient retention at 55-60 days gestation. However, a slight increase in nitrogen retention was observed for sows receiving 15 mg. This increase in nitrogen retention may be due to the increase in litter size in these sows. Sows receiving 15 mg of vitamin $B_6$ had similar plasma urea nitrogen levels as sows fed 1 mg. A previous study by Ritchie *et al.* (1960) saw no improvement when sows were fed supplemental vitamin $B_6$. However, the amount fed was only 5 mg and began 2 months after conception. Wohlbier and Siegel (1967*a* - as cited by ARC, 1981) found that litter size was slightly increased when vitamin $B_6$ in the total diet was increased to 17.2 mg. Easter *et al.* (1983) also saw an increase in litter size when gilts were supplemented with

vitamin $B_6$. Therefore, the increase in litter size in this study appears to be consistent with other studies. The increase in weaning-to-estrus interval may be linked to the influence of vitamin $B_6$ on steroid hormone regulation (Allgood and Cidlowski, 1991). Estrogen and progesterone are two hormones believed to be regulated by vitamin $B_6$ and are important in reproduction. Another explanation for the improved weaning-to-estrus interval is the decrease in backfat loss from weaning-to-estrus ($P=0.16$) in sows fed 15 mg of vitamin $B_6$. Therefore, sows fed 15 mg of vitamin $B_6$ were in better body condition at an early time compared to sows fed 1 mg of vitamin $B_6$. Whittemore *et al.* (1988) and Yang *et al.* (1989) found that breeding failure was usually associated with backfat depths of less than 10 mm at weaning. Sows in neither treatment had backfats of less than 10 mm, which may explain the observation that conception rates did not differ between the two diets.

Lean genotype sows had significantly lower backfat measurements during the postweaning and gestational periods and gestational weight gain was also lower in these sows compared to control sows. Since these lean genotypes have been selected for low backfat measurements, the differences observed were expected. Pond and Mersmann (1988) observed that lean genotype sows gained significantly less weight during gestation compared to obese sows. They suggested that the differences in weight gains was due to these lean genotypes requiring more energy for maintenance of the lean tissue mass. Despite the lower weight gains and backfats, the lean genotype sows in this study performed the same as controls in terms of reproduction. These results are similar to what another author observed. Vangen (1980 - as cited by Kerr and Cameron, 1995)

found that litter size and birthweights of piglets did not differ when pigs were selected on an index based on backfat and growth. Since nitrogen retention and plasma urea nitrogen levels were not different between the two genotypes, perhaps the energy requirements of these lean genotype sows were not being met by the diet and protein was being broken down and used as an energy source for maintenance.

Yorkshire sows retained less nitrogen, energy, and dry matter compared to Hampshire sows. However, no detrimental effects on reproduction were observed. In addition, nutrient intakes and digestibilities did not differ between the two breeds. PUN levels were significantly higher in Yorkshire sows indicating that protein was not being utilized efficiently by these sows. Yorkshire sows are also known to be leaner and tend to mature (reach full size) at a later date than Hampshire sows (Baidoo, personal communication). As stated earlier, diets were not adequate in energy or feed restriction caused increased protein catabolism in these sows.

No genotype x diet interactions were observed in this experiment. Thus indicating that lean genotype sows do not necessarily require more vitamin $B_6$ compared to non lean genotypes. However, significant breed x genotype interactions were observed for gestational weight gain, backfat at weaning and first estrus, and PUN levels at 99 days gestation. These interactions are most likely due to differences in the genetic potential of the genotypes and the breeds.

# CONCLUSION

The current study has revealed that the supplementation of sow diets with vitamin $B_6$ at 15 mg/kg of feed is beneficial for only certain reproductive parameters. Weaning-to-estrus interval was found to be significantly reduced by one day when sows were fed 15 mg of vitamin $B_6$ during the postweaning period. The level of vitamin $B_6$ did not significantly increase postweaning or gestational weight changes or backfat measurements, but sows supplemented at a higher level had better body conditioning. The proportion of pregnant sows to sows returning to estrus after breeding and the number of piglets born alive were not affected by supplementation above NAS-NRC (1988) recommendations. However, an increase of 0.7 piglets per litter was observed in sows fed 15 mg of vitamin $B_6$. Genotype of sows did not effect any reproductive parameters except backfat measurements and gestational weight gain.

Nitrogen retention was 5% higher for sows fed 15 mg of vitamin $B_6$ . The cause of this increase may be due to the slightly higher litter sizes in sows fed the higher level of vitamin $B_6$. Dry matter retention was significantly lower in sows fed 15 mg of vitamin $B_6$. These differences were due to a lower dry matter digestibility in these sows. No significant differences were observed between genotypes for any metabolic parameters measured. Breed differences in weaning weight change, weaning backfat, nitrogen retention, and PUN levels were observed. These differences are most likely due to breed genetic potential and nutrient utilization, but did not influence reproduction.

Although vitamin $B_6$ did not significantly improve reproduction and nutrient

retention in second parity sows, however, the improvement in weaning-to-estrus interval may require the supplementation of vitamin $B_6$ to sow rations.

Further research is suggested to:-

1. determine whether lower or higher dosages of vitamin $B_6$ will elicit similar or improved results.

2. determine whether there is a crucial period during postweaning or gestation when supplementation is more beneficial.

3. evaluate the relationship between hormonal status in returning to estrus of sows and vitamin $B_6$ levels in the diet.

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