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THE INFLUENCE OF GESTATIONAL DIETARY CALCIUM ON SERUM 1,25-DIHYDROXYCHOLECALCIFEROL IN SOWS AND THEIR BABY PIGS

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THE INFLUENCE OF GESTATIONAL DIETARY CALCIUM ON SERUM 1,25-DIHYDROXYCHOLECALCIFEROL IN SOWS AND THEIR BABY PIGS

By

Wuryastuti Hastari

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ABSTRACT

THE INFLUENCE OF GESTATIONAL DIETARY CALCIUM ON SERUM 1,25-DIHYDROXYCHOLECALCIFEROL IN SOWS AND THEIR BABY PIGS

By

Wuryastuti Hastari

Fifteen Yorkshire and crossbred sows were allotted to three groups of 5 sows, equalized for parity and fed corn-soy diets containing 0.5, 0.8 and 1.1% calcium, respectively, during gestation and lactation. Sera for 1,25-dihydroxycholecalciferol and mineral analyses (Ca, Mg, P, Cu and Zn) were obtained at 15 and 45 days of gestation, at parturition and at weaning. At parturition, colostrum samples for $1,25(OH)_2D_3$ analysis were collected and 5 piglets of each litter were randomly selected for study. Serum samples were obtained from these pigs at birth, at 10 and 21 days of age for $1,25(OH)_2D_3$ assays and to determine the relationships between maternal and neonatal minerals or $1,25(OH)_2D_3$ status.

In sows, serum $1,25(OH)_2D_3$ was significantly affected by dietary calcium within 15 days of initiating diets. During gestation and lactation, serum $1,25(OH)_2D_3$ correlated negatively ($r = -0.88$; $p < 0.05$) with serum calcium. Serum calcium was positively correlated ($p < 0.05$) with dietary calcium at days 15 and 45 of gestation and at farrowing. Serum magnesium was inversely related to serum calcium ($r = -0.49$; $p < 0.05$) during gestation and early lactation. In baby pigs, the mean serum $1,25(OH)_2D_3$ at birth was not affected by treatment but, by 10 days of age, the baby pig serum $1,25(OH)_2D_3$

correlated ($r = -0.62$; $p < 0.05$) with maternal serum calcium. Serum calcium and phosphorus increased significantly ($p < 0.05$) as maternal dietary calcium increased. The mean colostrum and serum $1,25(OH)_2D_3$ were significantly correlated ($r = 0.90$; $p < 0.05$) at parturition.

This study indicates that $1,25(OH)_2D_3$ production is quickly affected by changes in dietary calcium, but these changes did not have an in utero influence on the $1,25(OH)_2D_3$ status of fetal pigs.

Dedicated with love to

my mother, my father,

and my husband, R. Wasito

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INTRODUCTION

Rickets, as a consequence of vitamin D deficiency, is rarely found in modern swine operations in the United States. However, subclinical rickets may still be a problem in confinement rearing facilities in which the sows and piglets receive little ultraviolet irradiation, a more important factor for producing endogenous cholecalciferol than dietary vitamin D ingestion (Haddad, 1973). Besides that, the low plasma concentration of 25-hydroxycholcecalciferol (25-(OH)D_3) at birth (Horst and Littledike, 1982) and the rapid growth rate of the pig may predispose to neonatal rickets, a condition of vitamin D deficiency associated with abnormal skeletal growth and myopathy (Smith and Stern, 1967).

Realization that infants of vitamin D-deficient mothers have a great risk of developing rickets led researchers through a series of studies on finding the interrelationship between maternal and neonatal vitamin D status. Results of previous work with cattle (Goff et al., 1982), sheep (Barlet et al., 1978; Ross et al., 1979), rats (Noff and Edelstein, 1978) and humans (Hillman and Haddad, 1974) indicated that, at birth, there was a high correlation between maternal and neonatal plasma concentration of 25-(OH)D_3. However, neither the maternal and neonatal relationship nor the normal value of 1,25 dihydroxycholecalciferol (1,25$(OH)_2D_3$) in other domestic animals has been defined.

It is generally accepted that 1,25$(OH)_2D_3$ is the most active metabolite of vitamin D_3 (DeLuca, 1981; Holick et al., 1972b; Lawson et al., 1971). Studies in normal animals have documented that dietary

calcium (Ca) and phosphorus (P), parathyroid hormone (PTH) and the plasma concentration of $1,25(OH)_2D_3$ are important factors in regulating renal synthesis of $1,25(OH)_2D_3$ (Boyle et al., 1971; Garabedian et al., 1972; Galante et al., 1973). However, factors which enhance the production of $1,25(OH)_2D_3$ in various physiological states, such as gestation and lactation, have not yet been fully characterized. In a previous paper, Spanos et al. (1978) explained that there is no correlation between plasma concentration of $1,25(OH)_2D_3$ and plasma concentration of PTH and Ca during pregnancy and lactation in humans. A possible causal relationship between elevated prolactin and $1,25(OH)_2D_3$ has been suggested by Spanos et al. (1976) who demonstrated that injection of ovine prolactin into chicks can increase circulating concentration of $1,25(OH)_2D_3$. Studies in pregnant sows, on the other hand, have demonstrated that parenteral cholecalciferol treatment in the sows 20 days prepartum was an effective way of supplementing baby pigs with $25\text{-}(OH)D_3$ (via placental transport) and cholecalciferol (via the sow's milk), but not $1,25(OH)_2D_3$ (Goff et al., 1984).

We therefore decided to study the influence of gestational dietary Ca on serum concentration of $1,25(OH)_2D_3$ in sows and their baby pigs by feeding different concentrations of dietary Ca to the sows during gestation and lactation.

Many studies have indicated that the concentration of $1,25(OH)_2D_3$ in blood varies in different disease states such as parathyroid gland disorders (Broadus et al., 1980), sarcoidosis (Bell et al., 1979) or certain bone diseases (Rasmussen et al., 1980). Because changes in circulating $1,25(OH)_2D_3$ are of physiopathological importance in these diseases of Ca metabolism, the development of an assay

that is specific for and sensitive to $1,25(OH)_2D_3$ is really required. Several different assay techniques have been reported previously (Hughes et al., 1976; Horst et al., 1981; Dokoh et al., 1981; Manolagas et al., 1983). However, the methods frequently involve difficult extractions and require relatively large amounts of sample. In 1984, Reinhardt and co-workers developed a new assay for $1,25(OH)_2D_3$ which eliminated the use of HPLC for extraction and only needed 1 ml of serum. The assay was designed to quantitate $1,25(OH)_2D_3$ in serum from human subjects as well as experimental animals. However, we found the assay was capable of determining the concentration of $1,25(OH)_2D_3$ in colostrum.

The objectives of this research were: (1) to measure the serum concentration of $1,25(OH)_2D_3$ and Ca during gestation and lactation of sows fed a practical diet containing 0.5, 0.8 or 1.1% Ca during gestation and lactation; (2) to measure the serum concentration of $1,25(OH)_2D_3$ in neonatal pigs born to the above sows; (3) to determine the inter-relationships between maternal and neonatal $1,25(OH)_2D_3$ status in pigs; (4) to determine the inter-relationships between serum concentration of Ca and $1,25(OH)_2D_3$ during gestation and lactation in sows; and (5) to determine the influence of gestational dietary Ca on serum concentration of P, magnesium (Mg), copper (Cu) and zinc (Zn) in sows and their baby pigs.

LITERATURE REVIEW

INTRODUCTION

Vitamin D is the fat-soluble vitamin which is able to cure rickets (Hess et al., 1929). Two major forms of vitamin D are Vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). Both forms are considered to have the same activity biologically or nutritionally in most species except for chicks and New World monkeys which utilize D_3 more effectively than D_2 (Hunt et al., 1967; Steenbock et al., 1932; Holick et al., 1976).

Structurally, vitamin D_2 and D_3 have been identified as 9,10 secoderivatives of ergosterol (Askew et al., 1930) and 7 dehydrocholesterol, respectively (Windaus et al., 1936). The former is produced by the ultraviolet light irradiation of ergosterol synthesized in plants. In terms of antirachitic properties, partial irradiation of ergosterol with ultraviolet light has been considered to be better than complete irradiation (Steenbock et al., 1932). Vitamin D_3 is generated primarily in the malpighian layer of the epidermis by the action of 280-305 nm wavelength of ultraviolet light on 7 dehydrocholesterol (pro-vitamin D_3, an intermediate in cholesterol synthesis (Okano et al., 1977). The ability of the body to convert pro-vitamin D_3 to vitamin D_3 does not decrease with age (Mawer et al., 1982). Therefore, demonstration of a dietary requirement of vitamin D is unlikely in the presence of sufficient sun light.

HISTORICAL

The importance of sunlight for the normal skeletal growth had been speculated since ancient times (Hess et al., 1929). The soft skulls of Persians, who always wore turbans and covered most of their bodies, were used as evidence to support the above speculation (Hess et al., 1929). The earliest description of bone disease considered to be rickets was by two British doctors, Glisson and Whistler, in the middle of the seventeenth century (Orgler, 1953; Hess et al., 1929).

By the 1900's, the lesions of rickets were already described (Mellanby, 1919). In 1922, McCollum and his co-workers found that a substance in codliver oil, which has antirachitic properties, was actually different from vitamin A in its stability to heat and aeration. He then named the substance vitamin D (McCollum, 1912).

The structure of vitamin D from irradiation of food was determined almost simultaneously by Askew et al. (1930) and Windaus et al. (1932) who called it ergocalciferol (vitamin D_2). Several years after that, Windaus et al. (1936) also succeeded in identifying the structure of vitamin D_3 or cholecalciferol (Windaus et al., 1936).

Studies on the mode of action of vitamin D led to the information that there is a lag time between the administration of vitamin D and the initiation of the physiological action (Carlsson, 1952). The next observation led to one possible explanation for the time lag, that vitamin D may have to be further metabolized before it becomes active (DeLuca, 1976). In 1968, Blunt et al. succeeded in isolating the pure form of one of the metabolites of vitamin D_3 and identified it as 25-(OH)D_3. The biological activity of this compound was found to be 2 to 5 times greater than that of vitamin D_3 in healing rickets in rats.

At that time, it was thought to be the most active form of vitamin D_3. However, Haussler et al. (1968) reported that 25-(OH)D_3 was metabolized to a more polar compound. This metabolite could be obtained from the nuclear fraction of the chicken intestine and it had biological activity at least 13 times greater than cholecalciferol (Haussler et al., 1968). In 1972, that more polar compound was identified as 1,25(OH)$_2$-D_3 (Holick et al., 1972b). The fact that nephrectomized rats were unable to synthesize 1,25(OH)$_2D_3$ was used by Fraser and Kodicek in England as evidence that the kidney is the site of production of 1,25-(OH)$_2D_3$ (Lawson et al., 1971). Currently, 1,25(OH)$_2D_3$, the most active form of vitamin D_3, has been considered as a vitamin and a hormone (Holick et al., 1972b; Norman et al., 1977).

METABOLISM

Following its synthesis in the skin, the endogenous vitamin D_3 is transported to the liver by alpha 2-globulin of serum. Vitamin D_2 or supplemental vitamin D_3 in the diet is absorbed primarily in the distal part of the small intestine (jejunum and ileum). Bile salts are necessary for this absorption and chylomicra are responsible in transferring the absorbed vitamins D to the liver (Avioli and Haddad, 1973).

Studies on the absorption of radioactive vitamin D_3 in rats have revealed that about 72% of the vitamin D_3 recovered in thoracic duct lymph was associated with chylomicra while the remaining 28% was carried by alpha globulin (Dueland et al., 1982).

To function, vitamin D_3 must be activated metabolically through several sequential steps to a hormonal substance. The initial step of activation occurs in the liver in which vitamin D_3 is hydroxylated at

the C-25 position of the side chain to form 25-(OH)D_3, the major circulating form of vitamin D_3 (Holick and Clark, 1978). Although the liver is believed to be the major site of 25-hydroxylation in most species, Tucker et al. (1973) demonstrated that homogenates of kidney and small intestine from chicks are also capable of producing 25-(OH)D_3. This finding has been supported by Olson et al. (1976) who found that hydroxylation of vitamin D_3 at C-25 still occurs in hepatectomized animals. More recent studies indicate that other organs such as lung, pituitary glands, ovaries, adrenal glands and testes of rabbits and bovine have shown significant 25 hydroxylase activity (Ichikawa et al., 1983; Henry and Norman, 1984). The conversion of vitamin D_3 to 25-(OH)D_3 requires magnesium ions, NADPH, molecular oxygen, cytoplasmic protein and cytochrome P 450 (Bhattacharyya and DeLuca, 1974a; Yoon and DeLuca, 1980).

In vivo and in vitro experiments in rats suggested that 25-hydroxylase was regulated by a product feedback mechanism in order to prevent toxicity due to over-administration of vitamin D_3. Therefore, only limited amounts of vitamin D_3 can be hydroxylated to 25-(OH)D_3 (Horsting and DeLuca, 1969). These data have been supported by Bhattacharyya and DeLuca (1974b) who demonstrated that hepatic vitamin D_3 25-hydroxylation in chicks decreased twenty-four hours following the administration of physiological doses (20 IU) of vitamin D_3. However, additional investigation in rats did not support this concept (Rojanasathit and Haddad, 1976). Furthermore, Clark and Potts (1977) have recently shown in the vitamin D-depleted rats, which received different concentrations of dietary vitamin D_3 for either a week or three weeks, that the plasma concentration of 25-(OH)D_3 correlated with an

increase in vitamin D_3 intake irrespective of the dose of vitamin D_3 administered. Whether or not 25-hydroxylation of vitamin D_3 is subject to feedback regulation remains debatable.

After 25-hydroxylation, the 25-$(OH)D_3$ rapidly leaves the liver by binding to the plasma transport protein and goes to the mitochondria of renal tubule cells to be hydroxylated at either C-1 or C-24 position to form 1,25$(OH)_2D_3$ or 24,25$(OH)_2D_3$ (Holick and Clark, 1978; Henry and Norman, 1984). In states of hypervitaminosis D, 25-$(OH)D_3$ can also be hydroxylated at C-23 to yield 23,25$(OH)_2D_3$ or at C-26 to yield 25,26$(OH)_2D_3$ (Napoli et al., 1982; Napoli et al., 1981).

Hydroxylation at 1 alpha position is the very important step in vitamin D_3 metabolism to generate the most active form of vitamin D_3 which has the capability of stimulating either intestinal calcium transport or bone calcium mobilization (Boyle et al., 1972; Holick et al., 1972b; Wong et al., 1972). The 1-hydroxylation step is rate limiting and requires reduced nucleotide (NADPH), molecular oxygen and cytochrome P450 for enzymatic activity (Ghazarian and DeLuca, 1974).

The work of Boyle et al. (1971) has introduced the concept that serum Ca concentrations regulate the hydroxylation of 25-$(OH)D_3$ at the 1 alpha position. It has been known for some time that hypocalcemic conditions stimulate the production and release of PTH from parathyroid glands. It is now realized that this hormone acts in the kidney to stimulate the activity of 25-hydroxycholecalferol-1-hydroxylase and cause an increased production of 1,25$(OH)_2D_3$. However, under normocalcemic or hypercalcemic conditions, secretion of the PTH is minimal, the activity of 25-hydroxycholecalciferol-1-hydroxylase is also minimal and another metabolite of vitamin D_3, identified as 24,25$(OH)_2D_3$,

becomes predominant in circulation. The 24-hydroxylated metabolites are rapidly metabolized to $1,24,25(OH)_3D_3$ and excreted (Holick et al., 1972a; Rasmussen et al., 1972; Fraser and Kodicek, 1973; Garabedian et al., 1978).

The role of P depletion in the synthesis of $1,25(OH)_2D_3$ was studied following the discovery that thyroparathyroidectomized animals maintained on a low P and high Ca diet can still produce $1,25(OH)_2D_3$. Therefore, it was believed that low P diets controlled production of $1,25(OH)_2D_3$, even in the absence of PTH (Tanaka and DeLuca, 1973; Hughes et al., 1975; Haussler et al., 1977). Other in vivo investigations did not support this concept (Sommerville et al., 1978; Norman et al., 1977). These experiments provided evidence that P deprivation was much less effective in stimulating 1-hydroxylase activity than Ca deprivation. The degree of stimulation of 1-hydroxylase activity by low Ca diets is 10 times greater than the stimulation by low P diets (Rader et al., 1979; Harrison et al., 1982).

Hormones other than PTH have also been implicated in the regulation of the 25-OH-D_3-1 alpha-hydroxylase since many data indicate that, during gestation and lactation, there are remarkable shifts in concentrations of serum Ca without detectable changes in circulating PTH (Drake et al., 1979; Pitkin et al., 1979). Using the Japanese quail, it was demonstrated that both ovariectomy and anti-estrogen administration caused a reduction in renal production of $1,25(OH)_2D_3$ (Baksi and Kenny, 1976a). It has also been found that hypophysectomized rats have low sera concentrations of $1,25(OH)_2D_3$ and high serum concentration of $24,25(OH)_2D_3$. These conditions were reversed by the administration

of growth hormones for several days (Gray, 1981; Pahuja and DeLuca, 1981; Spencer and Tobiassen, 1981).

Extrarenal $1,25(OH)_2D_3$ synthesis is thought to occur because nephrectomized pregnant rats have been reported to be able to synthesize both $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$ (Weisman et al., 1979). Previous work with humans indicated that placenta, decidua, bone and fetal kidney are possible sites of $1,25(OH)_2D_3$ synthesis (Whitsett et al., 1981; Delvin et al., 1985; Howard et al., 1981).

FUNCTION

In terms of physiological function, vitamin D_3 and/or its metabolites are known to be responsible for maintaining Ca homeostasis which is important for normal calcification of bone, normal neuromuscular activity and normal muscle contraction (Norman, 1968; Tanaka and DeLuca, 1971; Omdhal and DeLuca, 1973; DeLuca, 1981).

To carry out that function, vitamin D_3 and/or its metabolites have three major effects. First, they initiate intestinal Ca and P absorption. This effect was first proven by Nicolaysen et al. (1953) who demonstrated that intestinal Ca transport in vitamin D-deficient rats was impaired. The process of intestinal absorption of Ca has been investigated in vivo using various techniques and all findings point to $1,25-(OH)_2D_3$ as the active metabolite (Schachter, 1963; Wasserman et al., 1961; Martin and DeLuca, 1969). Additional investigation indicated that $1,25(OH)_2D_3$ accelerates intestinal Ca absorption from all segments of the small intestine and from colon (Harrison and Harrison, 1969). Studies using thyroparathyroidectomized dogs have demonstrated that the effect on Ca absorption can be obtained solely by $1,25(OH)_2D_3$ (Schachter and Rosen, 1959).

The ultimate expression of vitamin D_3 action is thought to be at the intestinal brush-border membrane where the vitamin, in some way, increases diffusional-permeability of the microvilli to allow the entry of Ca ions into the cell. This perhaps can be manifested as a change in membrane structure and/or an effect on Ca carrier synthesis (Wasserman and Corradino, 1971; Omdhal and DeLuca, 1973; DeLuca and Schnoes, 1976).

The work of Steele et al. (1975) established that P transport can accompany the translocation of Ca. However, it is now apparent that vitamin D also stimulates P transport in the intestine in a manner which is different from the Ca process (Haussler, 1974).

A second effect of vitamin D_3 is to bring about the mobilization of Ca from previously formed bone to contribute to the Ca and PO_4 pool of the plasma (DeLuca, 1981). In vitro studies, using bone organ culture, have indicated that PTH is not involved in the process of Ca reabsorption from bone (Raisz et al., 1972). However, conflicting results have been obtained concerning the necessity of PTH in the action of $1,25(OH)_2D_3$ on bone resorption in vivo (Garabedian et al., 1974; Reynolds et al., 1976). From the studies of Tanaka and his co-workers, it is known that a form of vitamin D_3 other than $1,25(OH)_2D_3$ may also participate directly in the process of the Ca mobilization from bone (Haussler and Rasmussen, 1972; Wezeman, 1976; Bordier et al., 1977). This finding has also been supported by Reynolds et al. (1973) who demonstrated that, besides 1,25-dihydroxycholecalciferol, $25\text{-}(OH)D_3$ is also a potent stimulator of bone mineral resorption.

In terms of the mechanism of action by which vitamin D_3 promotes the mineral mobilization from bone, Wong et al. (1977) suggested that $1,25(OH)_2D_3$ induces bone resorption by activating osteoclasts and

inhibiting osteoblasts. In another investigation, Brommage and Newman (1979) explained that $1,25(OH)_2D_3$ stimulates the production of mineral "solubilizer" in the bone. This solubilizer reportedly binds to the surface of bone materials, increases their solubility and increases the flux from bone to the blood.

In addition to the effects in the intestine and the bone, vitamin D_3 is also believed to have a role in renal tubular mineral reabsorption (DeLuca, 1981). Studies on the effect of vitamin D_3 on renal Ca reabsorption indicated that $1,25(OH)_2D_3$ indeed improved renal reabsorption of this ion (Harris et al., 1976). However, since the experiments were done on animals with intact parathyroid glands (Gran, 1960) and since 99% of the filtered Ca is reabsorbed, even in vitamin D deficiency (Taylor and Wasserman, 1972), the direct action of vitamin D_3 on renal Ca reabsorption remains an enigma.

Regarding the possible role of vitamin D_3 on PO_4 reabsorption, Harrison and Harrison (1941) provided evidence that vitamin D_3 enhanced PO_4 reabsorption. Since PTH also causes phosphaturia, it is likely that this effect is mediated through suppression of PTH secretion by hypercalcemia rather than by a direct effect of $1,25(OH)_2D_3$ on the kidney (Puschett et al., 1972; Popovtzer et al., 1974). From the foregoing, it is clear that kidney, bone and small intestine are target tissues of vitamin D_3 in which the most potent metabolite of vitamin D_3, $1,25(OH)_2D_3$, acts by binding to specific, high-affinity receptors located in the cytoplasm of target cells.

In recent years, however, such receptors have been also identified in many other organs and tissues; among these are human leukocytes (Provvedini et al., 1983; Matsui et al., 1985), pancreas (Ishida et al.,

1983; Clark et al., 1981), skin (Hosomi et al., 1983), pituitary (Tornquist and Allardt, 1986), heart (Walters et al., 1986) and adrenal (Clark et al., 1986). Even though the exact role of $1,25(OH)_2D_3$ in diverse target organs has not been fully elucidated, it has been suggested that $1,25(OH)_2D_3$ may influence the proliferation of monocytes and T-lymphocytes (Provvideni et al., 1983; Matsui et al., 1985), differentiation of epidermal cells (Hosomi et al., 1983), secretion of insulin from pancreatic B cells (Ishida et al., 1983; Clark et al., 1981) and production of thyroid stimulating hormone from pituitary cells (Tornquist and Allardt, 1986).

VITAMIN D DEFICIENCY

Low or absence of vitamin D in the animal body is a classic cause of rickets. Other factors such as dietary lack of Ca or P or both also play an important role in the development of this disease (Chick et al., 1923; Harrison and Harrison, 1975). High levels of dietary iron can also induce P deficiency with subsequent development of rickets (Brock and Diamond, 1934). Based on these facts, rickets is a nutritional deficiency disease which is characterized by failure of adequate deposition of Ca and P in the bone. Rickets is often a climate-related disorder, too. Previous information indicated that it is limited largely to temperate latitudes (Orr et al., 1923). Rickets is considered to be a disease in young animals, in contrast to its counterpart called osteomalacia in adults (Nordin, 1960).

Stiffness of the forelegs is the first general sign of rickets in most animals. Nervousness and tetany may also appear quite early in animals with low blood Ca rickets. Swollen knee and hock joints, shortening

of muscles and tendons of the rear legs and scoliosis or curvature of the spine appear as the disease progresses in severity. All of these signs make animals unable to stand or walk comfortably for any period of time. In addition, enlargement of the costochondral junction can be easily palpated through the skin and is especially noticeable inside the thorax at the time of postmortem. These conditions persist even after healing. On the basis of the blood composition, the rachitic animals develop low blood Ca or low blood P or low blood concentrations of both Ca and P (Malherbe, 1956; Groth, 1958).

VITAMIN D TOXICITY

It is generally known that administration of large doses of vitamin D produces significant toxic effects. Deleterious effect is actually due to hypercalcemia resulting from excessive intestinal absorption of Ca and mobilization of Ca from bone, which then causes calcification of the arterial walls, intestinal walls and many other soft tissues such as heart, kidneys and lungs (Stanbury et al., 1980; Hughes et al., 1976; Davie and Lawson, 1980). Poisoning by vitamin D in dairy cows has resulted from prolonged prepartum administration of high doses of vitamin D_3 (30,000,000 IU/day) in order to prevent parturient paresis (Capen et al., 1966).

Enzootic calcinosis is a disease in cattle and other grazing animals manifested by widespread mineralization of soft tissues, especially in the cardiovascular and pulmonary systems. These signs are very similar to those of vitamin D toxicity. The disease has been reported from many countries under various names, but the characterization of the disease is known to be the same in all cases. Early experimental work has

indicated that enzootic calcinosis is associated with ingestion of plants belonging to the family Solanaceae (Worker and Carrilo, 1967; Gill et al., 1976; Singh et al., 1976). Additional investigations have determined that the plant (Solanum malacoxylon) contains an active substance that mimics the action of $1,25(OH)_2D_3$. It is effective in inducing calcium-binding protein (Ca-BP) synthesis and intestinal absorption of Ca and P in anephric, as well as in diabetic rats (Walling and Kimberg, 1975; Schneider et al., 1975). However, unlike the precursor of vitamin D_3, the active principle of Solanum malacoxylon is water soluble which makes it rapidly absorbed and excreted (O'Donnell and Smith, 1973).

There are other data indicating that vitamin D_3 sterols are also present in plant leaves of Cestrum diurnum, Dactylis glomerata, Medigo sativa and Trisetum flavescens. However, studies using HPLC and mass spectrophotometry have demonstrated that their vitamin D_3 activities are considerably less than Solanum malacoxylon (Krook et al., 1975; Peterlik et al., 1977; Morris and Levack, 1982; Horst et al., 1984).

Calcinosis in young pigs and young sheep can also be induced by giving these animals large doses of vitamin D_3 (Penn, 1970; Clegg and Hollands, 1976; Quarterman, 1964).

MATERIALS AND METHODS

ANIMALS

Fifteen sows, consisting of purebred Yorkshire, Yorkshire/Duroc crossbreds and Yorkshire/Landrace crossbreds, approximately averaging 175 kg in body weight, served as experimental animals. The sows were allotted to three groups of 5 sows equalized for parity, and the groups were assigned to diets containing 0.5%, 0.8% and 1.1% of calcium throughout gestation and lactation. The composition of the diets is presented in Table 1.

These sows were housed in an environmentally regulated, complete confinement, gestation facility with slotted concrete floors and were tied with neck collars and chains. The sows were fed 2.5 kg of grain/day and tap water ad libitum. At approximately 14 days before farrowing, the sows were moved into a facility equipped with metal farrowing stalls. The sows farrowed during November 1985.

The baby pigs were separated from the sows immediately after birth to obtain a presuckle blood sample. Each baby pig was weighed and had its tail docked, ears notched and needle teeth clipped. The baby pigs were also given parenteral antibiotic and iron dextran. Five piglets of each litter were identified by random selection to be followed on experiment through weaning.

BLOOD SAMPLING

Blood samples were collected into vacutainer[1] tubes from the anterior vena cava of the sows at 15 days and 45 days of gestation, at

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[1]Becton Dickinson, Rutherford, New Jersey

parturition and at weaning. Colostrum samples were obtained by manual milking during parturition.

Blood samples from baby pigs were obtained by vacutainer[1] tubes from the anterior vena cava at birth (colostrum deprived) and at 10 and 21 days of age.

For serum preparation, the blood samples were allowed to clot at room temperature (15 - 25°C) for at least 3 hours. All blood samples were centrifuged for 15 minutes at 760 x g. Each serum sample was withdrawn with a Pasteur pipette and placed into a 5 or 10 ml plastic vial. All serum and colostrum samples were frozen until assays were conducted.

ANALYTICAL PROCEDURES

Vitamin D analysis

The $1,25(OH)_2D_3$ in serum was quantified using a commercial radio receptor assay[2]. For sample extraction, 1.0 ml of each serum sample was pipetted into a 12x75 mm borosilicate glass tube. Fifty µl of ethanol buffer containing 1700 disintegrations per minute (DPM) [^{3}H] $1,25(OH)_2D_3$ were added to each serum sample and also to a scintillation vial containing 5 ml scintillation liquid[3] for calculating recoveries. One ml acetonitrile was added to each sample and each tube was then vortexed for 5 seconds and centrifuged for 10 minutes at 760 x g. While the samples were being centrifuged, Sep-Pak C-18[4] columns were

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[2] Immuno Nuclear Corporation, Stillwater, Minnesota.

[3] Research Product International Corp., Mount Prospect, Illinois.

[4] Waters Associates, Millford, Massachusetts.

Table 1. Composition of diets in this experiment

Ingredient	% Calcium 0.5%	0.8%	1.1%
Ground shelled corn	71.75	71.00	70.25
Soybean meal (44%)	14.50	14.50	14.50
Wheat bran	10.00	10.00	10.00
Calcium carbonate	0.50	1.25	2.00
Mono-dicalcium phosphate	1.50	1.50	1.50
Reg. T.M. salt	0.50	0.50	0.50
MSU VTM premix*	0.60	0.60	0.60
Vit. E-Se premix**	0.50	0.50	0.50
Choline chloride (60%)	0.15	0.15	0.15
	100.00	100.00	100.00

* Supplied the following per kg of diet: vitamin A, 3960 IU; vitamin D_3, 792 IU; riboflavin, 3.96 mg; d-pantothenic acid, 15.84 mg; niacin, 21.12 mg; vitamin B-12, 24 µg; vitamin K, 2.64 mg; choline chloride, 152.1 mg; zinc, 90 mg; iron, 71.3 mg; manganese, 4.5 mg; copper, 12 mg; iodine, 0.53 mg.

** Supplied 10 IU of vitamin E and 0.15 mg of selenium per kg of diet.

prepared by washing with 5 ml acetonitrile followed by two 5 ml washes with distilled water.

After centrifugation, the supernatant was poured off into another 12x75 mm glass tube containing 0.5 ml of 0.4 M potassium phosphate (pH 10.5) and vortexed for 5 seconds. This extract was then applied with a Pasteur pipette into the washed Sep-Pak C-18 columns. Excess salt and pigments were removed from the Sep-Pak C-18 columns by washing twice with distilled water and the interfering polar lipids were removed by washing with methanol:distilled water (70:30). The purified vitamin D metabolites were then eluted with 5 ml acetonitrile and the eluates were evaporated to dryness using a vacuum evaporator[5].

After the eluates had dried, each sample was reconstituted with 5 ml of hexane:isopropanol (98:2), mixed well by vortexing and applied to a Sep-Pak Silica[4] column. These columns were prepared before use by washing with 5 ml of isopropanol followed by 2 washes of 5 ml of hexane: isopropanol (98:2). Each eluate tube was rinsed with an additional 5 ml of hexane:isopropanol (98:2), vortexed for 5 seconds, and the rinse was applied to the Sep-Pak silica column. The 25-(OH)D_3 and 24,25$(OH)_2$-D_3 were removed from the Sep-Pak silica column by washing with 5 ml of hexane:isopropanol (96:4). The purified 1,25$(OH)_2D_3$ was eluted from the silica column with 5 ml of hexane:isopropanol (70:30) and dried in a vacuum evaporator.

The dried samples containing the 1,25$(OH)_2D_3$ fraction were immediately reconstituted with 200 µl ethanol buffer[2]. From this volume, 50 µl of purified sample were used to determine the recovery sample and two 50 µl aliquots were used for radio receptor assay.

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[5] Haake Buchler Instruments, Inc., Saddle Brook, New Jersey.

For assay, 50 ul of each $1,25(OH)_2D_3$ standard[2] (25, 50, 100, 200 and 400 pg/tube) and samples were added to the 12x75 mm borosilicate glass tubes. Four hundred ul of calf thymus receptor in phosphate-potassium chloride buffer[2] were added to each standard and sample. These mixtures were then vortexed for 5 seconds and incubated in a 15-20°C water bath for 60 seconds. At the end of this period, 11,500-13,500 DPM of [^{3}H] $1,25(OH)_2D_3$[2] in 50 ul ethanol buffer were added to each tube. The tubes were mixed well and incubated again for 60 minutes at 15-20°C. Following this incubation, the tubes were placed in an ice bath for 10 minutes to cool and then each tube received 100 ul charcoal suspension[2]. The tubes were vortexed for 5 seconds and placed for 20 minutes in an ice bath. After cooling, the tubes were vortexed again for 5 seconds and centrifuged for 15 minutes at 4°C at 1800 x g. Following the centrifugation, the supernatant was poured off into a scintillation vial containing 5 ml scintillation fluid[3]. The vial of supernate and scintillation fluid was mixed well by hand and then placed in a beta scintillation counter[6]. Data was expressed as mean counts per minute.

Minerals Analysis

Determinations of Ca, Mg, Cu and Zn concentrations in the blood serum were made by atomic absorption spectrophotometry[7]. In preparation for Ca and Mg quantification, each serum sample was pipetted into a 10 ml acid-washed flask and diluted 1:50 with 0.1% lanthanum chloride.

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[6] Model LS-800, Beckman Instruments, Inc., Fullerton, California.

[7] Model 5000, Perkin-Elmer, Norwalk, Connecticut.

For Cu and Zn quantification, each sample was diluted 1:1 and 1:5, respectively, with 1% hydrochloric acid in an acid-washed glass tube. Calcium standards used in this analysis had Ca concentrations of 1.0, 2.0 and 3.0 ug/ml, whereas the Mg, Cu and Zn standards contained 0.5, 1.0 and 2.0 ug/ml of the respective element. The standards and diluted specimens were then aspirated into an air-acetylene flame. Calcium was read at 422.7 nm, Mg at 285.2 nm, Cu at 324.7 nm and Zn at 213.9 nm.

The inorganic phosphorus (Pi) analyses were conducted in the Clinical Pathology Laboratory of the Veterinary Clinical Center at Michigan State University, using the Flexigem Centrifugal Analyzer[8]. Ten ul of each serum sample was pipetted into a cuvette and mixed well with 700 ul inorganic phosphorus UV reagent which contained 210 mM sulfuric acid and 0.40 mM ammonium molybdate. Serum inorganic phosphorus concentrations were determined directly with Flexigem spectrophotometer[8]. Absorption ratios were established by reading absorptions at 340 nm and 380 nm.

Statistical Analyses

The effects of dietary Ca treatments on serum $1,25(OH)_2D_3$ and minerals were evaluated by mixed design analysis of variance. One way analysis of variance and Dunnett's test were used to determine the significant differences between treatment means at each time. The interrelationships between maternal and neonatal concentration of minerals or $1,25(OH)_2D_3$ were determined by using correlation analysis. In this study, a difference was considered significant at the level of $P < 0.05$. All statistical analyses were performed by an IBM 4381 computer using the SAS[9] program.

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[8] Electro Nucleonics, Inc., Fairfield, New Jersey.

[9] Statistical Analytical System, Cary, North Carolina.

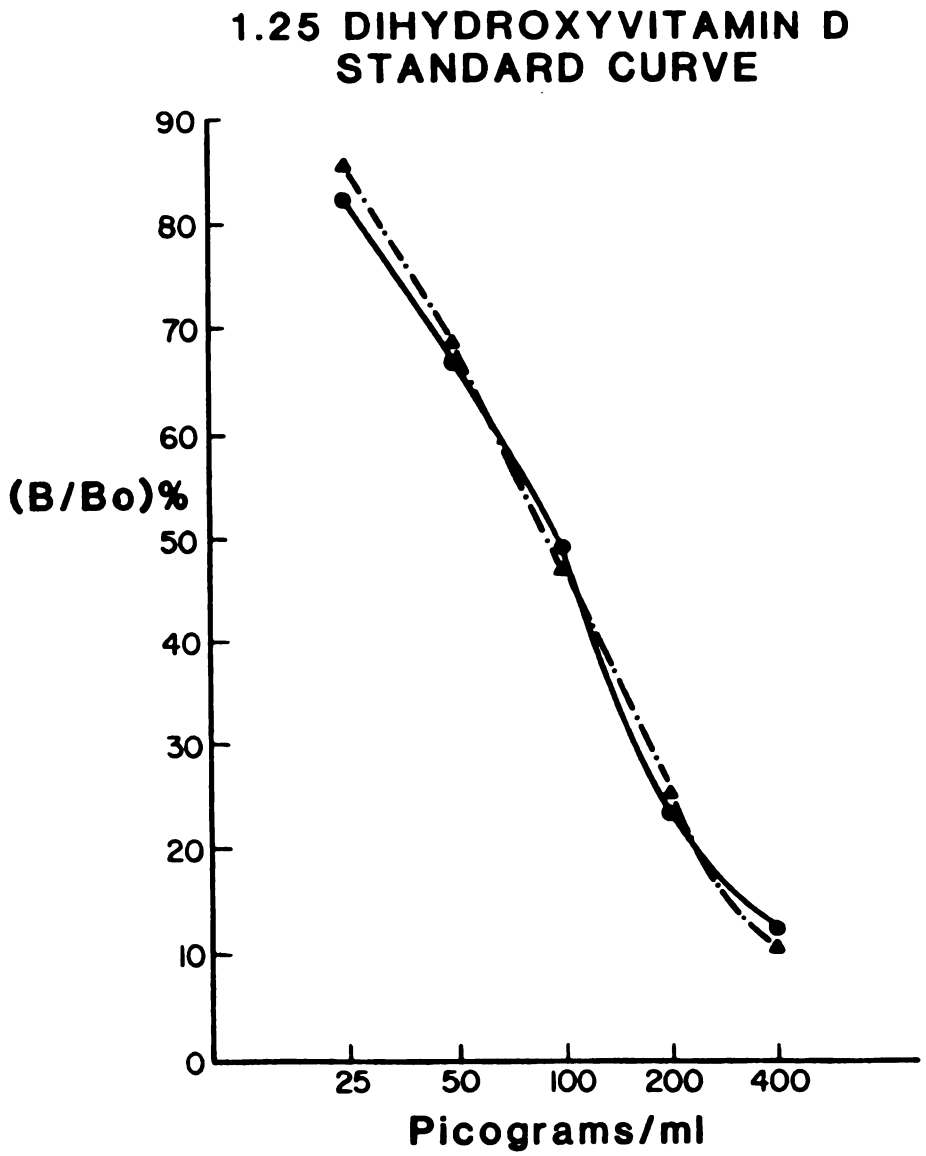

Fig 1. ●——● standard curve established by kit manufacturer.

▲·—·▲ typical standard curve derived from using the kit.

RESULTS

1,25 dihydroxycholecalciferol

The effect of dietary calcium on sow serum and colostrum 1,25 $(OH)_2D_3$ are presented in Table 2 and Figure 2. Within 15 days of initiating the different dietary Ca treatments, serum concentrations of $1,25(OH)_2D_3$ in sows were negatively correlated ($b = -.0.49$; $p < 0.05$) with dietary Ca (Fig. 3). As pregnancy progressed, serum $1,25(OH)_2D_3$ concentrations in all groups of the sows tended to increase until the time of farrowing. By weaning, the mean serum concentrations of $1,25(OH)_2D_3$ in all groups of the sows had decreased toward normal concentrations, even though the group means were still significantly different from each other ($p < 0.01$). At parturition, there was a significant correlation ($r = 0.90$; $p < 0.05$) between serum and colostrum concentrations of $1,25(OH)_2D_3$ and a significant negative correlation ($b = -0.40$; $p < 0.05$) between dietary Ca and colostrum $1,25(OH)_2D_3$ concentrations.

The effect of maternal dietary Ca on baby pig serum $1,25(OH)_2D_3$ are presented in Table 3 and Figure 5. The mean serum concentrations of $1,25(OH)_2D_3$ in the precolostrum piglets from the three groups were not significantly different, not correlated with maternal serum concentrations of $1,25(OH)_2D_3$, and tended to be inversely related to maternal dietary Ca. The mean serum $1,25(OH)_2D_3$ of the three groups of pigs at 10 days of age were more than twofold higher than the concentrations at birth, were significantly different ($p < 0.01$), were positively correlated ($r = 0.59$; $p < 0.05$) with maternal $1,25(OH)_2D_3$ concentrations and were negatively correlated ($r = -0.62$; $p < 0.05$) with maternal serum Ca. The mean concentrations of $1,25(OH)_2D_3$

of the groups of 21-day-old pigs were approximately 80-85% of the values observed 11 days before and did not correlate with maternal serum 1,25-$(OH)_2D_3$ concentrations.

Serum calcium

The effect of maternal dietary Ca on serum Ca in sows and their baby pigs are presented in Table 4. Serum Ca concentrations were affected by ($p < 0.05$) and positively correlated with ($r = 0.52$; $p < 0.05$) dietary Ca at days 15 and 45 of gestation and at farrowing, but only tended to be affected at weaning time. During gestation and lactation, serum Ca correlated negatively ($r = -0.88$; $P < 0.05$) with serum $1,25(OH)_2D_3$ (Fig. 4)).

Serum Ca in the baby pigs increased significantly ($p < 0.05$) as maternal dietary Ca increased at birth and at 10 and 21 days of age. There was negative correlation ($r = -0.55$; $p < 0.05$) between neonatal serum Ca and maternal serum 1, $25(OH)_2D_3$ concentrations.

Serum magnesium

The effect of maternal dietary Ca on serum Mg in sows and their baby pigs is presented in Table 5. Serum Mg in sows correlated negatively ($r = -0.49$; $p < 0.05$) with serum Ca during gestation and lactation except at weaning and did not correlate with serum $1,25(OH)_2D_3$ concentrations. In the baby pigs, serum Mg tended to be inversely related to serum Ca and P at birth, but not at 10 days and 21 days of age.

Serum phosphorus

The effect of maternal dietary Ca on serum P in sows and their baby pigs is presented in Table 6. No significant difference ($p > 0.05$) was observed in the mean serum concentrations of P in all groups

of the sows at any of the sampling periods during feeding. Serum P tended to be directly related to serum Ca and did not correlate with serum $1,25(OH)_2D_3$ concentrations. In the baby pigs, serum P increased significantly ($p < 0.05$) as maternal dietary Ca increased, and related directly to serum Ca concentrations.

Serum copper

Because of insufficient serum samples from the baby pigs, serum Cu concentrations were only determined on sows' serum. There were no significient overall Ca treatment effects on serum Cu in the sows throughout the experiment.

Serum zinc

In sows, serum Zn only tended to be inversely related to dietary Ca at day 15 of pregnancy. In the baby pigs, serum Zn was not influenced by maternal dietary Ca.

Table 2. Effect of dietary calcium on sow serum and colostrum $1,25(OH)_2D_3$ (pg/ml).

Time on feed (days)	Sample	n/group	Dietary calcium (%) 0.5	0.8[b]	1.1
15	serum	5	92.35 ± 1.70*[a]	70.25 ± 1.26	33.70 ± 1.44[a]
45	serum	5	107.94 ± 3.41[a]	77.24 ± 1.84	39.95 ± 2.60[a]
114 (parturition)	serum	5	124.52 ± 3.19[a]	104.63 ± 1.17	85.55 ± 2.63[a]
	colostrum	5	22.85 ± 1.92[a]	17.30 ± 1.04	13.93 ± 1.05[a]
140 (weaning)	serum	5	76.42 ± 3.84[a]	63.44 ± 3.10	58.70 ± 2.81[a]

* Values are means ± SEM

[a] Significantly different from control group ($p < 0.05$).

[b] Control group

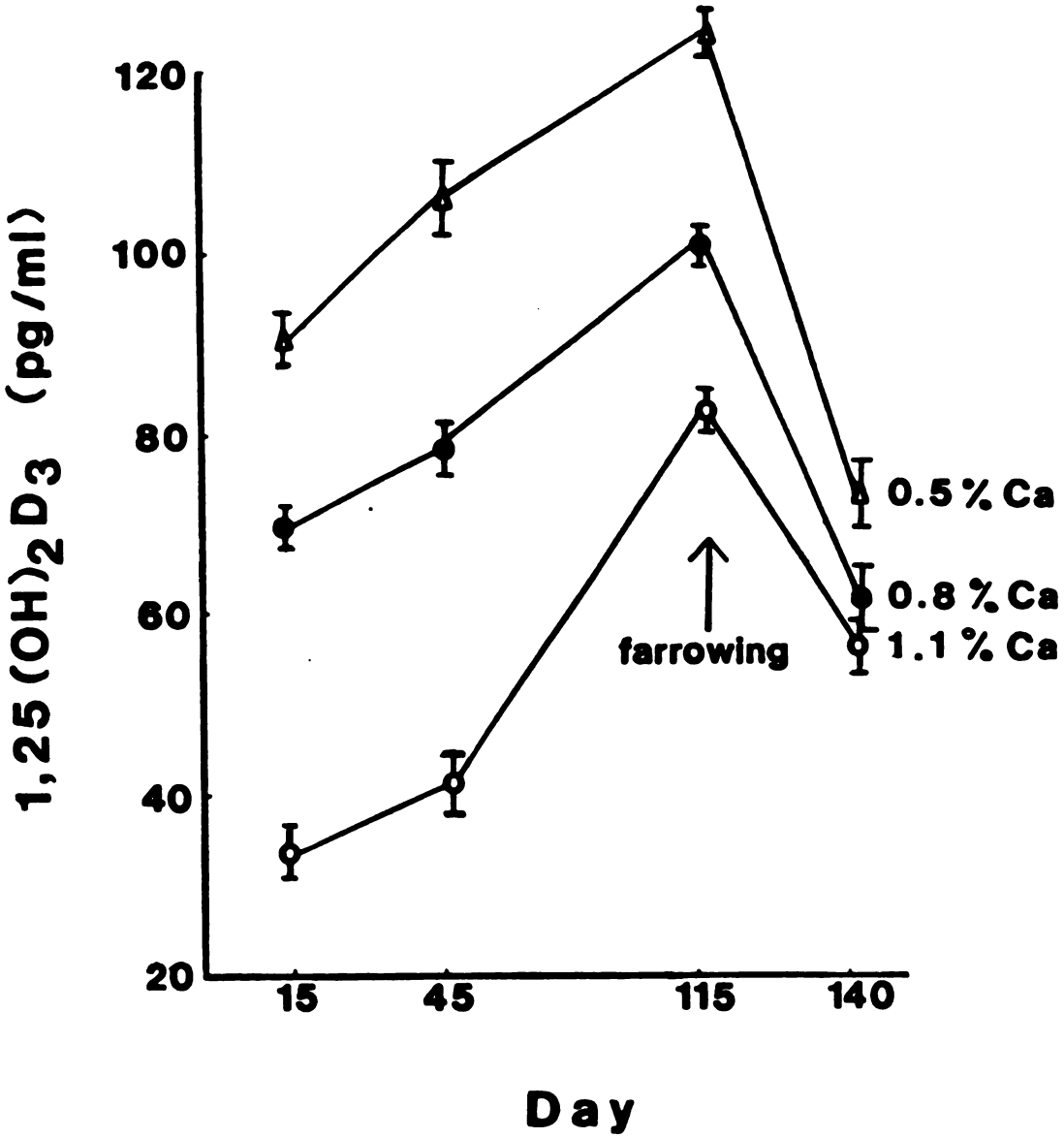

Figure 2. Effect of dietary calcium on sow serum $1,25(OH)_2D_3$.

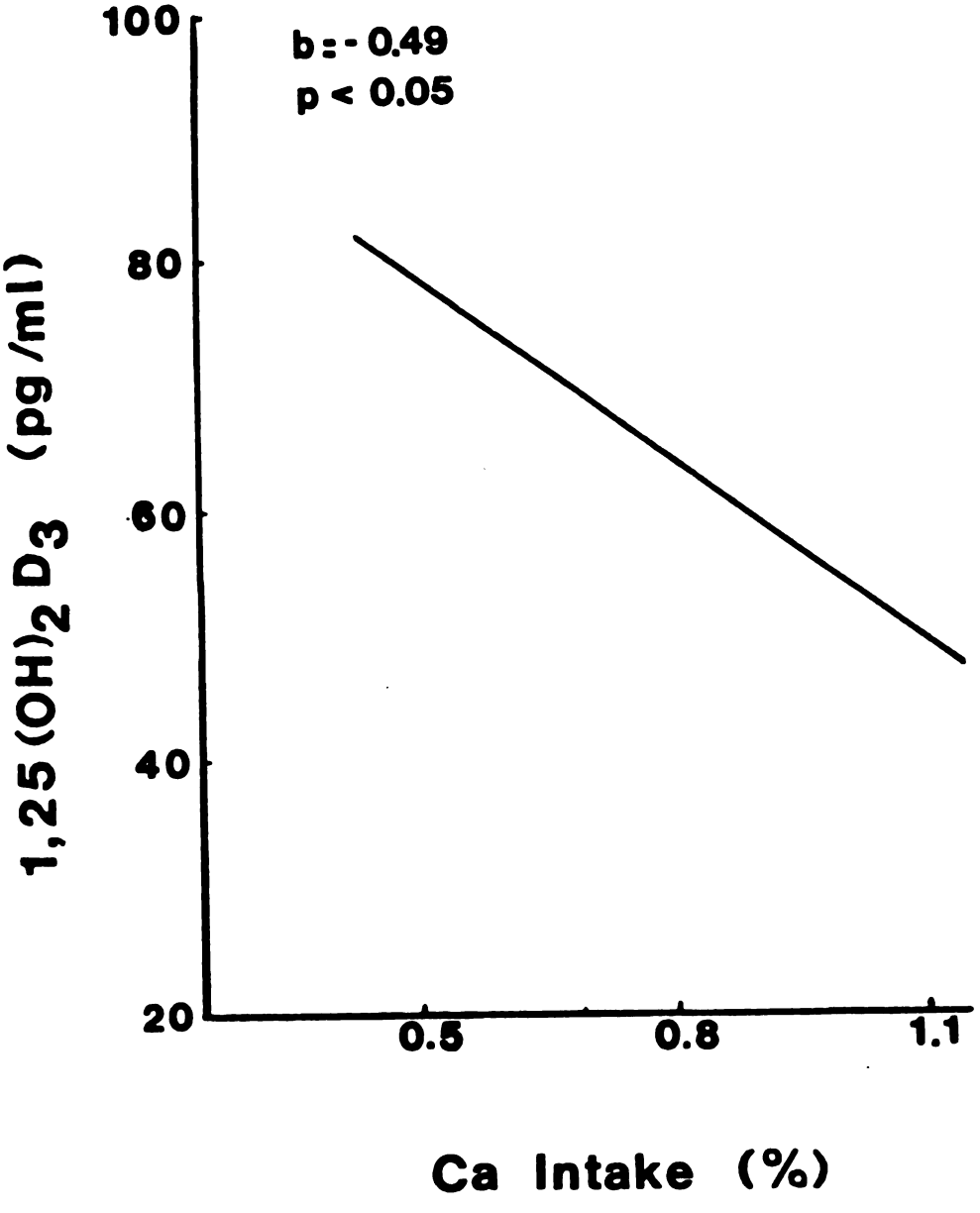

Figure 3. Interrelationships between serum $1,25(OH)_2$-D_3 concentrations and dietary calcium intake in sows.

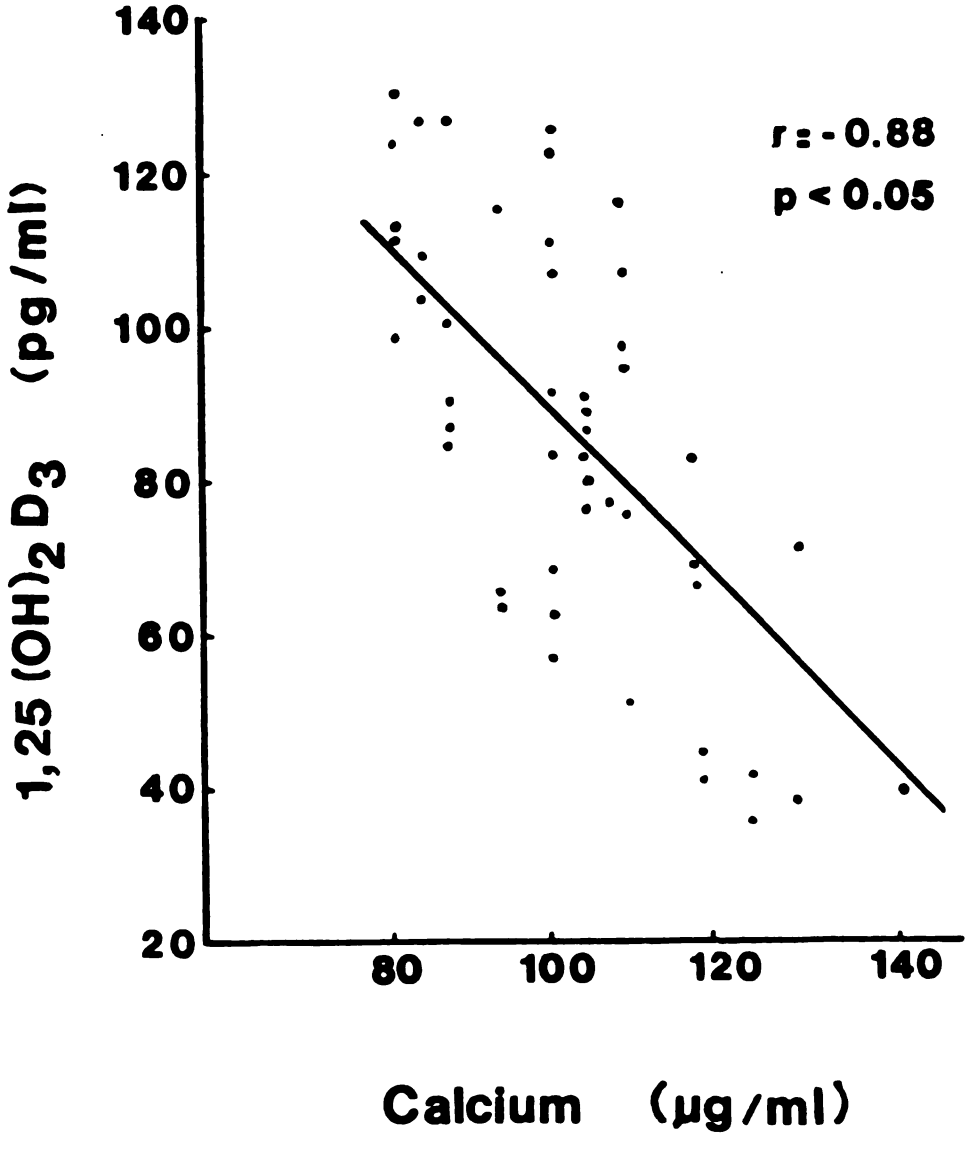

Figure 4. Interrelationships between serum 1,25-$(OH)_2D_3$ and serum Ca concentrations in sows.

Table 3. Effect of maternal dietary calcium on baby pig serum $1,25(OH)_2D_3$ (pg/ml).

Baby pigs age (days)	n/group	Maternal dietary calcium (%)		
		0.5	0.8[b]	1.1
0	25	44.16 ± 1.64*	43.47 ± 1.43	38.70 ± 0.74
10	25	112.52 ± 2.60[a]	94.65 ± 2.23	71.72 ± 2.65[a]
21	25	95.76 ± 1.64[a]	68.25 ± 1.01	58.62 ± 1.71[a]

* Values are means ± SEM

[a] Significantly different from control group ($p < 0.05$)

[b] Control group

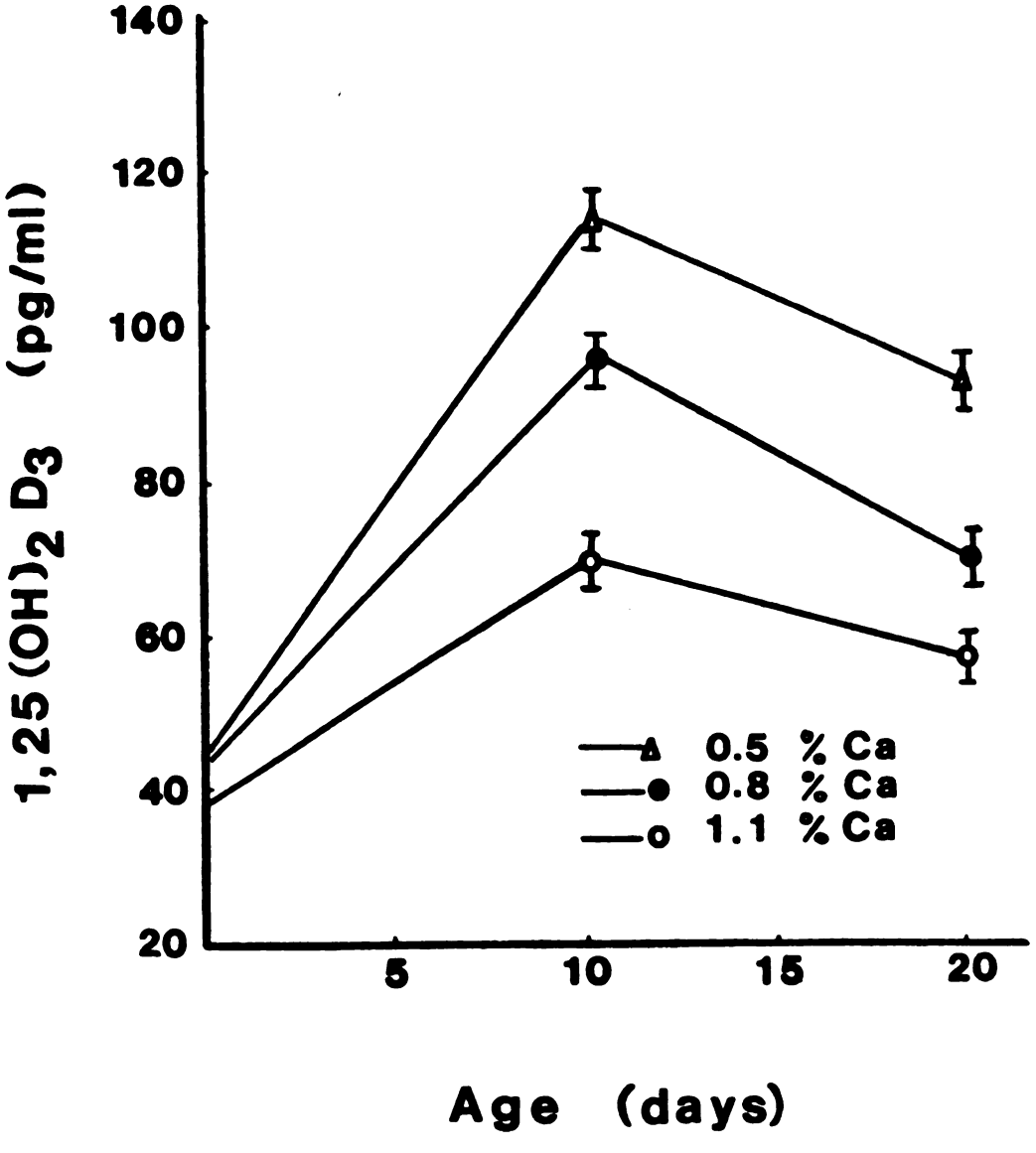

Figure 5. Effect of maternal dietary calcium on baby pig serum $1,25(OH)_2D_3$.

Table 4. Effect of maternal dietary calcium on serum calcium (µg/ml) in sows and their baby pigs.

Observation period	n/group	Maternal dietary calcium (%) 0.5	0.8[b]	1.1
Sows (days on feed)				
15	5	95.00 ± 6.45[a]	102.50 ± 5.95*	123.75 ± 2.39[a]
45	5	88.75 ± 4.30[a]	100.00 ± 5.00	118.75 ± 4.30[a]
114 (parturition)	5	83.75 ± 2.40[a]	93.75 ± 2.40	103.75 ± 5.54[a]
140 (weaning)	5	93.75 ± 3.75	101.25 ± 3.75	103.75 ± 3.75
Baby pigs (days of age)				
0	25	94.42 ± 2.71[a]	96.32 ± 2.97*	97.05 ± 2.37[a]
10	25	80.12 ± 1.82[a]	87.27 ± 1.35	102.80 ± 1.96[a]
21	25	97.05 ± 2.37[a]	102.80 ± 1.96	104.90 ± 2.68[a]

* Values are means ± SEM

[a] Significantly different from control group ($p < 0.05$)

[b] Control group

Table 5. Effect of maternal dietary calcium on serum magnesium (µg/ml) in sows and their baby pigs.

Observation period	n/group	Maternal dietary calcium (%) 0.5	0.8[b]	1.1
Sows				
days on feed				
15	5	23.75 ± 2.40[a]	20.00 ± 0.01*	18.75 ± 1.25[a]
45	5	22.50 ± 1.44[a]	20.00 ± 0.01	16.25 ± 2.40[a]
114 (parturition)	5	23.75 ± 2.40[a]	21.25 ± 1.25	17.50 ± 2.50[a]
140 (weaning)	5	27.50 ± 2.50	26.25 ± 3.75	25.00 ± 2.04
Baby pigs				
days of age				
0	25	23.12 ± 1.79	21.20 ± 1.67*	19.10 ± 0.71
10	25	22.89 ± 1.34	23.97 ± 1.37	22.30 ± 0.99
21	25	22.35 ± 0.92	22.17 ± 0.88	20.80 ± 0.44

* Values are means ± SEM

[a] Significantly different from control group ($p < 0.05$)

[b] Control group

Table 6. Effect of maternal dietary calcium on serum phosphorus (μg/ml) in sows and their baby pigs.

Observation period	n/group	Maternal dietary calcium (%) 0.5	0.8[b]	1.1
Sows				
days on feed				
15	5	61.50 ± 2.02	65.00 ± 2.34*	66.75 ± 4.30
45	5	58.25 ± 5.17	62.00 ± 8.97	62.25 ± 1.44
114 (parturition)	5	63.00 ± 0.41	71.75 ± 2.59	74.00 ± 8.40
140 (weaning)	5	74.75 ± 2.14	82.00 ± 5.84	83.75 ± 4.03
Baby pigs				
days of age				
0	25	62.60 ± 3.80[a]	75.16 ± 3.48*	72.65 ± 3.14[a]
10	25	64.27 ± 2.63[a]	70.51 ± 1.76	78.53 ± 2.63[a]
21	25	76.54 ± 3.34[a]	84.12 ± 2.65	87.90 ± 1.90[a]

* Values are means ± SEM

[a] Significantly different from control group ($p < 0.05$)

[b] Control group

DISCUSSION

Studies in normal non-pregnant humans or animals have documented that dietary Ca deprivation stimulates the renal synthesis of 1,25 $(OH)_2D_3$ and increases its serum concentration. These changes presumably are due to hypocalcemic stimulation of the parathyroids (Boyle et al., 1971).

During the mammalian reproductive cycle, pregnancy and lactation have been associated with alterations in maternal skeletal metabolism, Ca economy and its hormonal regulation. In pregnancy, the influx of Ca into the fetus for normal bone mineralization and development causes a significant decrease in the plasma concentration of Ca (Pitkin, 1979). In lactation, Ca movement from the plasma into the milk results in similar reduction in the concentration of calcium in the plasma (Tovered et al., 1976). For this Ca to be available to the fetus or to the milk, maternal absorption of Ca has to be increased. The principal regulatory factor in the absorption of Ca from the gut is vitamin D in its active form, $1,25(OH)_2D_3$ (Halloran et al., 1979).

A reduction in the serum Ca concentration, accompanied by elevation in the serum concentration of $1,25(OH)_2D_3$, was noted as early as two weeks post conception in sows fed a normal Ca diet. Further reduction in serum Ca and elevation in serum $1,25(OH)_2D_3$ concentrations were observed as pregnancy progressed. A significant negative correlation between the circulating levels of Ca and $1,25(OH)_2D_3$ was also observed in this study, which indicates that Ca also regulates the production of $1,25(OH)_2D_3$ during pregnancy. This corroborates a previous report in rats (Halloran et al., 1979).

The significant differences in serum $1,25(OH)_2D_3$ concentration among the groups of the sows within 15 days of initiating diets demonstrated that the production of $1,25(OH)_2D_3$ was quickly affected by dietary Ca. These observations demonstrate how sensitive $1,25(OH)_2D_3$ synthesis is to modest alterations in dietary Ca. Similar results were reported in normal non-pregnant cows (Blum et al., 1983) in which the increase in serum Ca of only 0.07 mmol/l (by $CaCl_2$ infusion over 24 hours) and a decrease in serum Ca of only 0.11 mmol/l (by Ca deprivation) were sufficient to respectively lower and raise plasma $1,25(OH)_2D_3$ levels.

As previously mentioned, extrarenal production of $1,25(OH)_2D_3$ was elucidated by experiments involving bilateral nephrectomized pregnant rats in which synthesis of $1,25(OH)_2D_3$ was not abolished (Weisman et al., 1979). Further investigations by Weisman and his coworkers (1979) have demonstrated that cultured explants of human decidua and placenta in vitro can synthesize $1,25(OH)_2D_3$ from $25\text{-}(OH)D_3$. They also found that the $25\text{-}(OH)D_3$-1 alpha-hydroxylase activity of the placental cells was significantly higher than that activity of the renal cells. Gray and his colleagues (1979) have provided evidence that homogenates of fetal rabbit and pig kidneys also synthesize $1,25(OH)_2D_3$ in vitro when the cells were incubated with $25\text{-}(OH)D_3$ in serum-free media. All these findings suggest that the site of $1,25(OH)_2D_3$ production during getation, besides the maternal kidneys, could be the fetoplacental unit.

Our observation that the serum concentration of $1,25(OH)_2D_3$ in all groups of the sows increased gradually throughout gestation might relate to the increased size of the fetoplacental unit as pregnancy

progressed. The reduction in the serum concentration of $1,25(OH)_2D_3$ after farrowing helps to confirm the fact that placenta and fetal kidneys are both involved in raising the maternal serum $1,25(OH)_2D_3$ during pregnancy. However, Pitkin et al. (1979) indicated that both prolactin and placental lactogen are responsible for an elevation in the maternal plasma $1,25(OH)_2D_3$ during pregnancy. These investigators also suggested that reduction in the levels of plasma $1,25(OH)_2D_3$ after delivery was due to the reduction in the levels of prolactin and placental lactogen. These variables were not investigated in this study.

There was no significant difference in concentration of $1,25(OH)_2D_3$ between groups of baby pigs at birth. This suggests that serum $1,25(OH)_2D_3$ of neonatal pigs was unaffected, in utero, by the dam's Ca intake. While we could not find any interrelationships between serum $1,25(OH)_2D_3$ of the sows and neonatal piglets, a high correlation between maternal and neonatal plasma concentrations of $1,25(OH)_2D_3$ was demonstrated in sheep (Ross et al., 1979), humans (Steichen et al., 1980) and in cattle (Goff et al., 1982). It is possible that this discrepancy is due to differences in placentation among species. It has also been suggested by Noff and Edelstein (1978) that $1,25(OH)_2D_3$ might be esterified by the rat fetus as a means of protection from high maternal concentrations of $1,25(OH)_2D_3$.

A significant correlation was found between maternal serum concentration of $1,25(OH)_2D_3$ and neonatal piglets serum concentrations of Ca which suggests that maternal $1,25(OH)_2D_3$ may affect the placental transport of Ca. Similar results have been demonstrated in cows by Goff et al. (1982) who postulated that the ability of the placenta to regulate

serum Ca and P concentrations in the fetus is partially dependent on the maternal $1,25(OH)_2D_3$ status.

In all groups of baby pigs, the serum concentration of $1,25(OH)_2D_3$ was two to three times greater at 10 days of age than at birth. This can be attributed to absorption of $1,25(OH)_2D_3$ from the sow's milk, which is essentially the sole source of nutrition for the baby pigs during their first 10 days of life. Another possibility is that the renal 25-$(OH)D_3$-1 alpha-hydroxylase activity in the baby pigs increases as pigs age, and it will respond to the level of Ca.

In this study we found that the amount of $1,25(OH)_2D_3$ in colostrum varied inversely with the amount of dietary Ca intake of the dam and was positively correlated ($r = 0.90$; $p < 0.05$) with serum concentration of $1,25(OH)_2D_3$. This finding supports the previous observation that the concentration of vitamin D in the circulation has a direct bearing on that in milk (Hollis et al., 1983) and expands our knowledge about the colostrum concentration of $1,25(OH)_2D_3$ in sows which had not been evaluated before.

In previous studies, Gray et al. (1979) have reported an inverse relationship between serum P and $1,25(OH)_2D_3$ in humans. However, in this porcine study, serum P was not significantly affected by dietary Ca intake and was not correlated with serum concentration of $1,25(OH)_2D_3$. It has been explained by Gray (1981) that low dietary Ca stimulates the production of PTH and $1,25(OH)_2D_3$ which mobilize both Ca and P from bone. Further action by PTH increases urinary P, consequently serum P concentration does not change. While the mechanism responsible for maintenance of nearly normal levels of P during Ca loading is not

apparent, the alterations in the concentrations of P clearly do not influence serum concentrations of $1,25(OH)_2D_3$.

The serum Mg concentrations were significantly affected by dietary Ca treatments ($p < 0.05$). The fall in serum Mg during Ca loading and the increase in serum Mg level during Ca deprivation are believed to be consequences of the alterations in serum PTH concentrations (Bethune et al., 1968).

According to Meintzer and Steenbock (1954), vitamin D status may have an effect on Mg absorption; however, these investigators explained that the effect of vitamin D on Mg absorption is not as direct as it is on Ca. The vitamin D effect on Mg may be due to its effect on the rates of accretion or mobilization of bone salt. In this study, we could not demonstrate any correlation between $1,25(OH)_2D_3$ and Mg concentrations and conclude that maternal dietary Ca does not have an in utero influence on the status of Mg of the fetal pigs.

It has been demonstrated that excess dietary Ca has a reducing effect upon the utilization of Zn in animals fed a corn-soy diet (Tucker and Salmon, 1955; Hoekstra, 1964). A significant effect of dietary Ca treatment on serum Zn concentration was not observed in this study. It is possible that the concentration of Ca in the diet was not high or low enough to produce any significant effect on serum Zn, or Zn supplementation in the diet was sufficient for maintenance of normal Zn concentration during Ca treatments.

There were no significant effects of dietary Ca treatments on serum concentration of Cu. It is believed that Ca does not have direct effect on Cu status but may do so indirectly via the alterations in Zn metabolism.

CONCLUSIONS

The competitive binding radioreceptor assay for $1,25(OH)_2D_3$ used in this study was found very satisfactory for swine serum and colostrum. Using this assay, serum $1,25(OH)_2D_3$ of normally fed, multiparous sows can be expected to approximate 70 pg/ml at the time of breeding, to increase during gestation to about 104 pg/ml by parturition and to decline during lactation to approximately 63 pg/ml by weaning. Colostrum of these sows is expected to contain approximately 17 pg of $1,25-(OH)_2D_3$/ml. Serum $1,25(OH)_2D_3$ concentrations (pg/ml) of baby pigs from normally-fed sows on a corn-soy based diet can be expected to approximate 43 pg/ml prior to suckling, 94 pg/ml at 10 days of age and 68 pg/ml at 3 weeks of age.

In the pregnant sows, serum $1,25(OH)_2D_3$ concentrations responded quickly and inversely to modest changes in dietary Ca. The negative correlation between dietary Ca and $1,25(OH)_2D_3$ persisted through gestation and early lactation. Based upon the serum $1,25(OH)_2D_3$ data of newborn pigs, this parameter was unaffected by the dam's dietary Ca intake, but was especially influenced by the $1,25(OH)_2D_3$ content of the colostrum. It was concluded that the $1,25(OH)_2D_3$ status of the sows does not have an in utero influence on the fetal $1,25(OH)_2D_3$ status.

Of the other nutrients (Mg, P, Cu, Zn) observed in this study, maternal dietary Ca changes caused significant alterations on the maternal serum Mg concentrations during gestation and early lactation. These

changes did not have any correlation with serum $1,25(OH)_2D_3$ concentrations. In baby pigs, serum Ca and P increased significantly as maternal dietary Ca increased. However, the correlation between serum Ca and P concentrations in baby pigs and maternal serum $1,25(OH)_2D_3$ was only observed at the time of birth.

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