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EFFECTS OF ELEVATED DIETARY ENERGY AND PROTEIN DURING LATE GESTATION ON MAMMARY DEVELOPMENT IN GILTS

By

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ABSTRACT

Effects of Elevated Dietary Protein and Energy During Late Gestation on Mammary Development in Gilts.

By

William C. Weldon

Thirty-two gilts were used to determine the effects of elevated dietary energy and crude protein during late gestation on mammary development. On d 75 of gestation, gilts were randomly assigned in a 2x2 arangement to adequate (5760 kcal ME/d;AE) or elevated (10,500 kcal ME/d;EE) energy and adequate (216 g CP/d;AP) or elevated (330 g CP/d;EP) protein. On d 105 of gestation gilts were sacrificed and total mastectomies performed. Mammary tissue was separated into parenchymal (PCM) and extraparenchymal stroma (STR) tissue and analyzed for DNA, RNA, protein and lipid. No significant interactions between energy and protein existed. When equalized for number of mammary glands and maternal body wt, PCM wt was 27% greater (P=.02) in AE gilts than in EE gilts, but energy level had no effect on STR wt. Total PCM DNA was 29.8% (P=.03) greater in gilts fed AE verses gilts fed EE (P=.03). Total PCM RNA (P=.02) and total PCM protein (P=.01) were greater in gilts fed AE compared to gilts fed EE. Dietary protein level did not affect any mammary variables measured except for the tendency of EP to reduce STR weight (P=.08). We conclude that EP between d 75 and d 105 does not benefit mammary development, while EE is detrimental.

I would like to dedicate this thesis to my parents for their love and support throughout my life and during the completion of this degree. Without their examples of hard work and perseverance, this thesis could not have been completed.

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INTRODUCTION

In an effort to improve the efficiency of pork production, each year more emphasis is put on the role of the sow in a typical production system. As time has advanced, the pork industry has concentrated on increasing the number of pigs raised per sow per year. The dam's milk is the sole source of nutrients for the neonatal pig for at least two weeks after birth in most production systems. During this period, starvation accounts for 50% of preweaning deaths in healthy herds (England, 1986). The ability of the sow to produce nutrients for its offspring is dependent upon the ability of the mammary gland to produce milk. Therefore, the mammary gland plays a key role in the success and failure of a commercial swine operation. It is surprising, however, that little research has been conducted on the mammary gland of swine, and how nutritional management can influence its optimal development. Limited research has been conducted on the pattern of mammary development in swine (Hacker and Hill, 1972; Kensinger et al., 1982; 1986b) but a review of the literature finds few studies that have tried to determine how this development may be manipulated (Kensinger et al., 1986a).

My study was designed to determine the effects of elevated dietary energy and protein during late gestation on

development of the mammary glands of gilts. Because it is impossible to affect only mammary development, the effects of diet on other factors involved in the reproductive performance of sows were measured, namely weight of the sow and fetus and carbohydrate storage in the fetal liver. This information should help us to better understand how dietary energy and protein affect these components of reproduction.

REVIEW OF LITERATURE

Introduction

Reproductive and lactational performance of sows remains crucial to the economic success and failure of swine production. Nutrition affects the reproductive performance of sows but how these effects are mediated is less well understood. Nutrition may act on the sows ability to maintain body weight and fruitful reproductive status, provide nutrients to the developing fetuses, or produce nutrients for the neonate. The following review of literature will address these issues. This review will pay particular attention to effects of dietary energy and protein levels during pregnancy on development of the gilt, her fetuses and mammary glands.

NUTRITIONAL EFFECTS ON THE SOW AND FETUS

Effects on the Sow

The effects of varying feed intake during pregnancy have been studied by many researchers (Baker et al., 1969; Agricultural Research Council, 1981; Cromwell et al., 1982; Pond et al., 1985; Pond et al., 1986). In these studies treatments generally consisted of increasing total feed intake

of the sow. Generally, these studies considered that the response obtained was due to increased levels of energy, but all components of the diet were increased. Treatments among these trials varied greatly from severe feed restrictions (2,000 kcal DE/day, Pond et al., 1985; 1986) to feeding levels substantially above requirements (1.36 kg extra feed per sow per day, Cromwell et al., 1982), but most studies used more moderate levels of feed intake (Baker et al., 1969). Similar results on sow condition have been observed in most cases, but differences vary directly with feeding level. In general, increasing feeding level during gestation increases sow weight gain quadratically, followed by a linear increase in weight loss during lactation. Backfat thickness of sows during gestation increased as feeding level increased.

The ability of gilts to maintain pregnancy during severe energy restriction has been documented (Pond et al., 1985; 1986). This phenomenon could be related to the concept that pregnant gilts have the ability to gain weight at significantly lower feeding levels than non-pregnant gilts, or the concept of "pregnancy anabolism". Pregnancy anabolism was suggested by the data of Salmon-Legagneur (1964), Heap et al. (1967) and Lodge (1972). However, this concept is disputed by other researchers (Hovell et al., 1977; Shields and Mahan, 1983; Close et al., 1984; Pond et al., 1986). These researchers contend that increases in body weight of pregnant gilts over non-pregnant gilts fed the same level of feed at similar ages, is due, nearly entirely, to increases in the weight of the conceptus and mammary tissue.

The optimal level of feeding or energy intake of pregnant gilts remains disputed. It has been suggested by O'Grady (1980) that weight gains during pregnancy should be from 10 to 40 kg for optimal reproductive performance. Optimal reproduction is defined as the ability of sows to maintain pregnancy without becoming excessively fat or losing large amounts of maternal body tissue during lactation (O'Grady, 1980). Verstegen (1987) contends that gilts should be fed to gain a total of 45 kg during gestation, of which 20 kg would be gained in the weight of the products of conception, and 10 kg would be lost during the subsequent lactation, for a net gain between each successive parity of 15 kg. A feeding level that results in these body weight relationships should provide a level of nutrition that provides maximum conservation of weight and body condition of sows during lactation. Thus increasing lifetime productivity of the sow (Close and Cole, 1986).

Another nutritional aspect of the pregnant gilt is her requirement for amino acids. There is no requirement for crude protein per se, but crude protein in the diet must supply indispensable amino acids and total amino nitrogen (National Research Council, 1988). Researchers have looked at many different protein levels and protein sequences in diets of pregnant sows and gilts (Holden et al., 1968; Pond et al., 1968; Baker et al., 1970; Hammel et al., 1976; Greenhalgh et al., 1977; Mahan, 1977; NCR 42 Committee on Swine Nutrition Research (NCR-42), 1978; Shields et al., 1980; Shields et al.,

1985). Research indicates that sows under moderate dietary protein restriction have satisfactory reproductive performance, as measured by number of pigs born and litter weight, but weight gain of sows during gestation is lower. Shields and Mahan (1983) have shown that gilts on a 14% CP diet throughout their reproductive cycle have a net increase in maternal protein and fat during gestation. Sows under severe dietary protein restriction during gestation (5% CP) deposit more maternal fat, but less protein, lose less weight during lactation (Shields et al., 1985), and have decreased feed intake during the following lactation (O'Grady, 1980) than sows fed at a higher protein level (14% CP; Shields et al., 1985). Interpretation of data from studies on the protein requirement during gestation is complicated due to an interaction between gestation and lactation dietary protein levels (Mahan and Mangan, 1975; NCR-42 Committee on Swine Nutrition, 1978). Indeed, many of the detrimental effects of moderately low protein levels (9 to 11%) fed during gestation, can be overcome by feeding higher protein levels during lactation (>15%) (NCR-42 Committee on Swine Nutrition, 1978).

The research discussed above describes effects of feeding level (energy) and dietary protein on body weight gain and performance of the sow. These factors may also affect the reproductive performance of sows by affecting the fetus.

Effects of Maternal Nutrition on the Fetus

The sow is the sole source of nutrients to the developing fetus. Therefore, nutrition of the sow, along with

her ability to provide nutrients during nutrient restriction are key to survival and development of the fetus.

High feeding level during early gestation may reduce embryo survival in gilts (Frobish and Steele, 1970; Dyck and Strain, 1980). In a more recent study Toplis and Genesi (1983) stated that high feeding levels in early gestation did not jeopardize survival of the embryo in multiparous sows. This conclusion is supported by Heap et al. (1967) and Brooks and Cole (1971). The results are thus, inconclusive as to the effects of feeding level during early gestation on survival of the embryo.

The percentage of maternal energy intake used for growth and maintenance of reproductive tissues is low (3%) at d 50 of gestation (Noblet et al., 1985). Therefore, during this early period of development, increasing dietary energy above the requirement has little effect on the development of the conceptus. The energy requirement of reproductive tissues increases as pregnancy advances and growth of the fetus increases. At d 110 of gestation, 12% of maternal energy intake is required for reproductive processes (Noblet et al., 1985). This would indicate that the energy requirement of pregnant gilts increases as gestation progresses. The demand for energy is greatest during the final 30 d of gestation. It is during this period that fetal development is maximal (Noblet et al., 1985). This has led the Agricultural Research Council (ARC; 1981) to recommend gradual increases in the feed allowances of gestating sows as pregnancy progresses. Results

from studies utilizing this type of feeding regimen have been mixed. Studies indicate that there is no effect of feeding level on number of pigs born, (Lodge et al., 1966; Baker et al., 1969; ARC, 1981; Whittemore et al., 1984) even under severe energy restriction (Pond et al. 1985, 1986; 2,000 kcal DE/d). However, in one study by Cromwell et al. (1982) increasing feed intake from 1.82 to 3.2 kg/d in the final 23 days of gestation increased the number of pigs born. Generally, there was a quadratic effect of energy level on birth weight, with birth weight increasing as energy level increased, up to approximately 6 Mcal DE/sow/d. In contrast increasing feeding level beyond 6 Mcal DE/sow/d has little effect on birthweight (NRC, 1988).

Noblet et al. (1985) established that as pregnancy progresses the percent of maternal protein intake required for reproduction increases, being low in early gestation (7% at d 50) and increasing with development of reproductive tissues and fetuses (41% at d 110).

Protein level during gestation has little or no effect on the number of pigs born (Holden et al., 1968; Baker et al., 1970; Greenhalgh et al., 1977; Mahan, 1977; NCR-42 Committee on Swine Nutrition, 1978; Shields et al, 1980; 1985). Severe dietary protein restriction however, decreases pig birth weight (Pond et al., 1968; Hammel et al., 1976; Shields et al, 1980; 1985). Therefore, dietary protein plays a small role in the development of the fetus when dietary protein is adequate or slightly restricted.

The evidence suggests that nutrition of the sow

affects development of the fetus. For effects to be expressed however, restrictions have to be very severe in most cases. The severity of restriction required to produce a response may be due to factors such as the variability of the response criteria and the gestation by lactation interaction, but is probably due to the ability of the sow to compensate for dietary restrictions by catabolizing maternal body stores (Swick and Benevenga, 1977).

In summary one can conclude that increasing energy in the diet does not effect the number of pigs born but may increase birth weights. Dietary protein does not effect either the number or weight of pigs unless gilts are fed diets severely deficient in protein.

Effects of Maternal Nutrition on Glycogen Storage by the Fetus

Pigs are born with little to protect themselves from their environment. They have sparse pelage and small energy stores at birth (Mersmann, 1974). Pigs are born without brown fat and thus they have a reduced ability to regulate body temperature when compared to those species with brown fat (Bruk, 1970). Neonatal pigs are, therefore, limited to carbohydrate metabolism for their primary source of energy (Mersmann, 1974). The pig is born with carbohydrate stores found primarily in muscle and liver (Dawes and Shelly, 1968). This carbohydrate is stored during late gestation and is rapidly metabolized 12 to 18 h after birth (Mersmann et al, 1972). Glycogen metabolism is critical during this period

for regulation of body temperature and survival of the neonate.

Maternal nutrition has had varying effects on carbohydrate stores in the piglet. Seerley et al. (1974) found that glycogen stores of the fetus were increased when sows were fed additional energy in the form of cornstarch from d 109 of gestation to parturition. Yet, neither Boyd et al. (1978) or Okai et al. (1978) found any effect of additional dietary energy on glycogen storage when sows were supplemented with either cornstarch or tallow. Alloxan-induced diabetes beginning at d 70 of gestation increases fetal liver glycogen stores (Ezekwe and Martin, 1978). In a more recent study fasting and refeeding did not influence hepatic glycogen storage (Yen et al., 1982). Thus nutrition's effect on fetal liver glycogen storage is not well defined. It would appear that maternal nutrition does not readily affect carbohydrate storage by the fetus.

Effects of Nutrition During Pregnancy on Subsequent Lactational Performance.

The mammary gland and its secretion play crucial roles in decreasing piglet mortality because starvation accounts for 50% of all preweaning mortality in healthy herds (England, 1986). The effects of feeding level during gestation on subsequent milk production are difficult to quantify because there is an interaction between gestation and lactation feeding levels (Mahan and Mangan, 1975; NCR-42 Committee on

Swine Nutrition, 1978). The effects of feeding level are also difficult to quantify because variables used to measure milk production in swine, namely litter weight gain, is not a direct measure of milk production. Litter weight gain explains only 34% of the variation in milk yield (Lewis et al., 1978). This causes researchers to use large numbers of animals to achieve statistical significance, making experiments labor intensive and expensive.

Effects of feed intake during pregnancy on lactational performance (as measured by litter weight gain) have been studied by many researchers. Lodge et al. (1966) found no effect of feed intake during pregnancy on litter weights at 21 d or at weaning. In a comprehensive study Baker et al. (1969) found that increasing gestation feeding level from .9 kg to 2.4 kg feed per sow per day linearly increased weaning weight as maternal feed intake increased. Increasing feed intake during the final 21 d of gestation increases weaning weight and survivability (Cromwell et al., 1982). Reese (1985) suggested that additional energy during the latter periods of gestation may increase preweaning survivability in herds where survivability is less than 85%, a similar conclusion was reached by Pettigrew (1981). Therefore, it would appear that increasing gestation feeding level is beneficial to subsequent lactational performance, particularly during the final weeks.

The effects of protein level during gestation on lactational performance of gilts has been studied by many workers. Shields, (1980) and the NCR-42 Committee on Swine Nutrition (1978) found no effect of gestation protein intake

on litter weight at 4 d of age. On the other hand Greenhalgh et al. (1977) found that gilts given more protein during gestation had heavier litters at 3 wk of age. Mahan (1977) found that low dietary protein during gestation inhibited milk secretion during the first week of lactation. These experiments indicate that low dietary protein during gestation may adversely affect milk production of gilts.

From the reviewed experiments it seems that lactational performance is enhanced when gilts are fed elevated energy and protein during the latter stages of pregnancy. The component of reproductive performance affected is not clear. Increases in litter weaning weights may be an expression of heavier birth weights or an increased ability of the mammary gland to produce milk. The effects of nutrition on the development of the mammary gland will be discussed in the following sections.

Hormonal Control of Mammary Development

Growth and development of the mammary gland is mediated by the endocrine system. This control has been extensively reviewed elsewhere (Tucker, 1981; 1985). In general, estrogens promote duct growth while progesterones promote lobulo-alveolar growth (Tucker, 1985). These two steroid hormones synergize with each other to produce rapid growth of the mammary gland during pregnancy. It appears that the absolute quantities are more important to mammary development than the ratio of estrogen to progesterone (Tucker, 1985).

Growth hormone and prolactin have been shown to be mammiogenic. If one administers growth hormone to heifers it

will stimulate mammary growth (Bauman, 1984). Similarly, prolactin has been shown to cause local proliferation of mammary tissue (Tucker, 1985). Because concentrations of growth hormone and prolactin do not normally change during periods of rapid mammary growth, it has been postulated that these hormones play a role of permission in mammary development. The role of absolute quantities of these hormones in circulation in respect to mammary development has yet to be defined. Insulin and the thyroid hormones must also be present in minimum concentrations for the mammary gland to develop at a normal rate but their concentrations are not thought to be limiting under normal circumstances (Tucker, 1985).

The Pattern of Mammary Development

Before one can quantify mammary development at different stages of life, it is important to identify a method of measurement that quantifies total milk secreting tissue. The use of deoxyribonucleic acid (DNA) as an estimate of mammary secretory tissue was first reported by Kirkham and Turner (1953). DNA per mammary cell nucleus is constant during pregnancy and lactation (Tucker and Reese, 1962) and therefore DNA should give an indication of the number of secretory cells present. Nucleic acid content of mammary tissue is highly correlated (r=.85) with subsequent milk yield (Tucker, 1966). This procedure is not flawless, however, because other cell types within the mammary gland also contain DNA, including fat

cells, and connective tissue. Measurements of nucleic acids can be combined with measures of lipid as well as connective tissue to give a better indication of the components of mammary development.

As stated above, total DNA can be used to estimate mammary cell number because DNA per mammary cell is constant. Ribonucleic Acid (RNA) is involved in protein synthesis, therefore an increase in RNA would indicate an increase in the ability of the mammary gland to produce proteins. An increase in the ratio of RNA to DNA would be indicative of an increase in the amount of machinery available to produce protein per cell, or the cells functional activity.

Other measures can be used to quantify development of mammary tissue, including mammary gland weight, volume, area, histometric methods, incorporation of tritium and magnetic resonance. These procedures each have their own problems. Measures of gland weight, area and volume do not take into account differences in cell types or composition of the mammary tissue. Incorporation of tritiated thymadine is expensive and results in disposal of animals. Magnetic resonance is a fairly new technique but it is limited to small animals and equipment is expensive and not readily available.

Development from Birth to Conception

Little is currently known about the development of swine mammary glands during this phase of growth. This is due to common rearing practices. Generally, gilts are not selected for breeding purposes until just prior to puberty. Before

selection, gilts are housed with growing-finishing pigs. This method of rearing is practiced because the percentage of gilts retained for breeding is low, there are no economic benefits of special housing, and producers can collect growth information which may be important in predicting growth rates of progeny. Due to this lack of information on mammary development in prepubertal swine other species will be discussed.

At birth the mammary gland is a system of immature ducts and fatty stromal tissue (Tucker, 1987). In the rat and heifer this ductile system elongates into the stroma (fatty and connective tissue) and grows at a rate similar to general body growth (Sinha and Tucker, 1969b) during early life.

Before puberty, the mammary gland undergoes a period of growth which is faster than the female's general body growth (Astwood et al., 1937). This increase is associated with the onset of puberty. Hormones produced by the ovary are involved because ovariectomy inhibits this growth response (Cowie, 1949). It has also been found by Muldoon (1979) that specific estrogen receptors appear in the mammary gland near puberty in mice. Allometric growth continues in rats and heifers through several estrous cycles before returning to an isometric growth pattern until conception (Sinha and Tucker, 1969a).

Development from puberty to conception is an isometric period of growth with most development occurring during estrus. There is little development during the luteal phase of the estrous cycle (Sinha and Tucker, 1969a; 1969b). This

return to an isometric growth pattern may be caused by the asynchrony of estrogen and progesterone secretion during the estrous cycle (Tucker, 1987). Development of the mammary gland between puberty and conception is small in the species studied (cattle and rats) and its importance to mammary development in swine is limited since most gilts are bred on their second estrus.

Development from Conception to Parturition

True alveoli are not formed until after conception (Tucker, 1987). Upon conception, ducts elongate, alveoli form and replace portions of the mammary fat pad (Tucker, 1987).

In cattle, mammary development increases exponentially at a rate of 25% per month throughout gestation (Swanson and Poffenbarger, 1979). In contrast, swine have little development of the mammary gland through d 50 of gestation (Hacker and Hill, 1972; Kensinger et al., 1982) followed by a massive proliferation of mammary cells between d 50 and d 100 of gestation (Hacker and Hill, 1972). A similar pattern was observed in DNA concentration of a mammary biopsy by Kensinger et al. (1982).

This allometric growth phase may be associated with the fact that between d 60 and d 90 of gestation development of uterine and placental tissue is complete, and estrogen output by the uterus increases some 400 fold (Knight et al., 1977). There is also an increase in circulating estrogen concentrations during this period (Dehoff et al., 1986). During this same period of development there is no increase in

plasma concentration of prolactin or growth hormone (Dehoff et al., 1986). Thus, estrogen may be driving the rapid mammary growth between d 75 and d 90 of gestation.

Development During Lactation

In many species mammary development continues through early lactation. In goats total mammary DNA continues to increase through d 21 of lactation (Knight and Peaker, 1984), and in cattle there is a 65% increase in total mammary DNA between d 10 prepartum and d 10 postpartum (Ackers et al., 1981).

In swine, Hacker (1972) found that total DNA/mammary gland did not increase between farrowing and day 2 of lactation. Kensinger (1982) found no difference in DNA concentration of mammary gland biopsies between d 90 of gestation and d 4 of lactation. Therefore, mammary gland development during lactation in the pig may be complete before onset of lactogenesis, but further study involving more animals is required. This is similar to the pattern of development in sheep (Anderson, R.R., 1975).

Nutritional Effects on Mammary Development

Nutrition affects the function of endocrine glands (Jackson, Cit Pyska, 1979) because acute or chronic starvation, caloric restriction and vitamin and mineral deficiencies impair endocrine secretion. Since growth of the mammary gland is mediated by these secretions, nutrition may greatly affect growth and development of mammary tissue.

Birth to Puberty

Effects of prepubertal feeding level have been intensely studied in cattle. Swanson (1960) and Gardner et al. (1977) found that increasing the feeding of dairy heifers during rearing, decreased subsequent milk production. High feeding levels decreased total DNA and weight of parenchymal tissue of cattle (Uhrin et al., 1986; Sjersen et al., 1982) and sheep (Johnsson and Hart, 1985). This reduced growth is caused by an effect of nutrition on hormone concentration in the plasma. High feeding levels have been associated with depressed concentrations of growth hormone in cattle (Sjersen et al., 1982) and sheep (Johnsson et al., 1985). Lambs fed ad libitum that received daily injections of .1 mg bovine pituitary growth hormone did not have reduced total mammary DNA when compared to animals restricted in feed intake (Johnsson et al., 1986). Total DNA in the mammary glands of lambs was highly correlated with growth hormone concentration (r=.95) (Johnsson et al., 1986). In a similar study heifers given daily injections of growth hormone had increased mammary parenchymal weight (Bauman, 1984); thus, concentrations of growth hormone during the prepubertal allometric growth phase influence mammary development in these animals.

Nutrition between puberty and conception does not influence mammary development in cattle (Sjersen et al., 1982) or in rats (Singh and Turner, 1971).

Dietary protein level affects mammary development in rats. Rats receiving protein restricted diets had less total mammary DNA than rats receiving adequate dietary protein (Pyska and Styczynski, 1979; Singh and Turner, 1971). It is suggested by Singh and Turner (1971) that this decrease in development is due to the effect of protein restriction on thyroid hormones and FSH secretion. Secretion of FSH ultimately effects the sensitivity of the mammary gland to estrogens and progesterone (Griffith and Turner, 1961; Moon, 1962) in rats. In a more recent study, rats fed diets at NRC requirements rather than high or low levels of protein (Chew et al., 1984) were found to have increased mammary DNA.

During Pregnancy

Mammary development during pregnancy is quantitatively the most important stage of development. Detailed studies on nutritional-hormonal-mammary development interactions during pregnancy have not been performed. Few data are available for total DNA or other quantitative measures of mammary development.

Mahan (1977) suggested that gilts restricted in dietary protein have reduced early lactational performance during the first week postpartum. Noblet et al. (1985) suggested that increasing energy from 20 MJ/d to 30 MJ/d during gestation increases gross mammary weight. Twin-bearing ewes fed a low plane of nutrition during pregnancy had reduced linear dimensions of the udder (Mellor, 1985; Mellor et al., 1987). These ewes had lower plasma GH concentrations 32 and 25 d

prepartum (Mellor et al., 1987). Food restriction in pair-fed rats decreased mammary gland weight, protein and DNA contents as well as RNA to DNA ratio (Naismith and Robinson, 1983).

Summary

With the information available to date, and the subjectivity and problems associated with interpreting linear measurements and gross weight it is hard to draw strong conclusions about the effect of nutrition during pregnancy on mammary development. More understanding may come from greater knowledge of hormone concentrations during pregnancy and how they might be affected by nutrition.

More research is needed to fully explain effects of nutrition during pregnancy on mammary development. This lack of information is surprising because development of the mammary gland is great during this period.

MATERIALS AND METHODS

Animals

Animals selected were comprised of purebred Yorksires and crossbreds that were at least one half Yorksire. Purebreds and crossbreds were randomly assigned across treatment and balanced according to ancestry. Gilts from different rearing groups were removed from the finishing floor, mixed and transported to synchronize estrus. At this time, gilts were vaccinated for parvovirus, leptospirosis, and erysipelas. Gilts were treated for internal and external parasites. Gilts were exposed twice daily to a mature boar to identify those gilts in estrus. Once identified in estrus, gilts were bred on two consecutive days or until they refused to stand for the boar. The day of the last mating was considered the first day of pregnancy. All gilts within a group were bred within 4 d.

Management

After breeding an entire group, gilts were weighed and moved to an environmentally controlled building and housed in individual stalls. Gilts were fed once daily a diet containing NRC (1979) requirements for protein, energy and all other nutrients (Table 1). Throughout the early gestation period (d 1 to d 75) and late gestation (d 75 to d 105) gilts were

weighed and tenth rib backfat measurements were taken weekly.

Treatment

On d 75 ± 2, gilts were assigned to 1 of 4 dietary treatments. Gilts received one of two levels of crude protein (CP; 216 or 330 g/d) and one of two levels of energy (5760 or 10,500 kcal ME/d). Therefore, the four dietary treatments in this study were: 216 g/d CP and 5,760 kcal ME/d (AA); 216 g/d CP and 10,500 kcal ME/d (AE); 330 g/d CP and 5,760 kcal ME/d (EA); 330 g/d CP and 10,500 kcal ME/d (EE). Dietary protein level was manipulated by changing concentrations of corn and soybean meal in the diet (Table 1). Dietary energy level was changed in a similar fashion and by addition of cornstarch. Daily allotment of feed per head per day was also varied to achieve dietary treatments. Daily intake of vitamins and minerals was held constant. Lysine intake was held constant across treatments of like protein level. During early gestation gilts were fed once daily followed by feeding twice daily after treatments were imposed at d 75 of gestation.

Slaughter

On d 105 ± 2 of gestation, animals were stunned and exsanguinated. After exsanguination, the mammary gland was removed, placed in a plastic bag and immersed in a mixture of dry ice and acetone until frozen. Glands were subsequently stored at -21°C until analyses were performed.

The utero-placental complex was removed and weighed. Individual fetuses were weighed, position and sex recorded and

euthanatized. Livers from the five most cervical fetuses per sow were removed, placed in plastic bags and immediately frozen in dry ice and acetone. The empty utero-placental complex was weighed after removal of fetuses and fluid. Carcass weight was recorded immediately after slaughter and before carcasses were chilled to -4 C. On the day following slaughter, backfat and loin eye area were measured at the tenth rib.

Dissection

Frozen mammary glands were cut into 1.5 cm slices. Right side glands were separated into parenchymal and extraparenchymal stromal tissue, free of skin. These tissues could be distinguished by differences in color. Extraparenchymal stromal tissue was white, and the parenchymal tissue was pink in color. Tissues were weighed and stored at -21°C until homogenization.

All tissues from the right side of the mammary glands were immersed in liquid nitrogen for 5 min and forced through a half inch grid. These frozen crumbles were then homogenized at high speed for 30 seconds in a Waring blender at -20°C until all tissue was a fine powder. The powderd sample was mixed and a representative sample was obtained from this frozen powder.

Chemical Analysis

All chemical analyses were performed on both the parenchymal and extraparenchymal stromal tissue.

Nucleic Acids

RNA and DNA were determined by the method of Tucker (1964). Duplicate 1 g samples of frozen, powdered tissue were weighed into a 30 ml beaker, 19 ml of cold deionized distilled water was added, and tissue was suspended with a polytron TM[1] at top speed for five seconds. Two ml samples of homogenate were weighed into a 15 ml Corex centrifuge tube and suspended in 8 ml of 95% ethanol for 24 h at room temperature. Samples were extracted for 24 h with 9 ml of methanol:chloroform (2:1/v:v) and for another 24 h with 9 ml of anhydrous ether under constant agitation. The samples were extracted twice with 5 ml of ice cold 10% tri-chloracetic acid (TCA). TCA was removed by washing with ice cold 95% ethanol saturated with sodium acetate. Samples were digested in 2 ml of 1 N KOH for 15 h at 37°C, and the digest acidified with .3 ml ice cold 6 N HCl and 5 ml ice cold 10% perchloric acid (PCA). The residue was washed twice with 5 ml 5% PCA and combined supernatants analyzed for RNA ribose using the orcinol procedure. The remaining residue were extracted with 5 ml of 10% PCA at 70°C for 15 min and washed twice with 5 ml of 10% PCA. Combined supernatants were then analyzed for DNA by measuring light absorbance at 268 nm using a Beckman DB spectrophotometer[2]. Yeast RNA and highly polymerized DNA were used as standards for RNA and DNA respectively[3].

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[1]Brinkman Instruments, Westburg, New York.
[2]Gilford Instrument Laboratories, Inc., Oberlin, OH.
[3]Sigma Chemical Company, St. Louis, MO.

Protein

Protein was determined using the method of Lowry (1951). Approximately .1 g of homogenate was weighed into a test tube and diluted. One ml of the resulting mixture was used for determination of total protein. Bovine serum albumin was used as the protein standard[3].

Lipid

Lipid was determined by collection of extracts from RNA and DNA procedure in a aluminum pan and allowing organic solvents to evaporate leaving lipid. Pans were dried at 60 C for 12 hr and difference in weight was considered lipid content of the sample.

Glycogen

Glycogen was determined using a modified Seifter (1949) procedure. Approximately 1 g of liver was weighed into a test tube and 3 ml of 30% KOH was added. Tubes were then boiled for 20 min. The sample was quantitatively transferred into a 50 ml volumetric flask and brought to volume. One tenth ml of this dilution was added to a test tube with 9.9 ml deionized distilled water. One ml of diluted sample was added to a test tube and placed in an ice bath until samples were cold. Two ml of .2% anthone[4] in 95% sulfuric acid was added to tubes while being bathed in the ice bath. Samples were vortexed and returned to the ice bath. Samples were boiled in a water bath for 10 min and immediately cooled.

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[4]Sigma Chemical Co., St. Louis, MO.

Absorbance was determined using a Beckman DU Spectrophotometer at 620 nm. Anthone (.2%) was used in the reference cell. Standards were obtained from mammalian glycogen.[5]

Statistical Analysis

Data from this factorial experiment were subjected to a analysis of variance using the general linear models procedures of the Statistical Analysis System. Main effects were protein and energy levels. Preliminary statistical analysis found no effect of breed, period or number of fetuses, and therefore all data were pooled for analysis.

[5]Boehringer-Manneheim Biochemicals, Indianapolis, IN.

TABLE 1. COMPOSITON OF EXPERIMENTAL DIETS

	Diet			
Ingredient, kg	AA	AE	EA	EE
Corn	853.6	722.5	685.7	931.5
Soybean meal, 44%	100.8	13.0	270.1	43.1
Mono-dicalcium phosphate	18.5	9.2	15.7	4.8
Ground limestone	12.2	7.6	12.3	9.9
MSU vit-TM premix[a]	5.0	3.5	6.2	2.8
Vit E-SE premix[b]	5.0	2.9	5.0	2.8
Salt	5.0	2.9	5.2	3.4
Corn starch	0.0	236.5	0.0	0.0
L-lysine HCL	0.0	1.0	0.0	2.4
	1000.0	1000.0	1000.0	1000.0
Calculated analyses				
ME, kcal/kg	3139.4	3339.6	3124.4	3212.2
Crude protein, %	11.9	17.9	10.4	10.1
Lysine, %	.5	.3	.9	.5
Ca, %	.8	.5	.8	.5
P, %	.7	.4	.7	.4
Supplied daily/head				
Feed, kg/d	1.8	3.1	1.8	3.3
ME, kcal/d	5760.0	10500.0	5760.0	10500.0
Crude protein, g/d	216.0	218.1	330.0	330.0
Lysine, g/d	9.1	9.1	17.4	17.4

[a]Composition per kg premix was Vitamin A, 661,380 IU; Vitamin D, 132,276 IU; menadione, .66 g; riboflavin, .66 g; niacin, 3.53 g; D-pantothenic acid, 2.64 g; choline chloride, 88.18 g; Vitamin B12, 3.96 mg; Zn, 7.5 g; Mn, 7.5 g; I, .11 g; Cu, 2 g; Fe, 12 g.

[b]Composition per kg premix was Vitamin E, 3310 IU; Se, 19.8 mg.

RESULTS

Three gilts were sacrificed on d 75 of gestation to assess mammary development at the time when treatments were imposed. Characteristics of the mammary glands of gilts at d 75 and d 105 are listed in Table 2. These data support the contention that the period of time between d 75 and d 105 of gestation is a period during which a tremendous amount of mammary development occurs. It should be noted that gilts killed on d 75 were only used as a reference point, no statistical analysis was performed on these data.

Mammary Development

Analysis of the data found no significant interactions between energy and protein. Therefore, only main effects will be reported.

Protein level had no effect ($P>.05$) on parenchymal weight per gland, but elevated (E) dietary protein tended to decrease the weight of the extraparenchymal stromal tissue on a per gland basis ($P<.09$) compared to gilts fed adequate (A) protein. Elevated levels of energy tended ($P<.08$) to depress parenchymal weights but had no effect on the weight of the extraparenchymal stroma (Table 3). When differences were adjusted for maternal weight (weight of the sow less the

weight of the fetuses), the deletarious effects of elevated energy on parenchymal weight became more apparent (P<.02).

The effects of energy and protein on the total nucleic acid, protein and lipid of the parenchymal tissue per functional mammary gland are listed in Table 4. The level of protein had no effect on any of the components measured. Gilts fed elevated levels of energy tended to have less DNA (P<.08) and significantly less RNA and total protein than gilts fed adequate levels of energy, when results were compared on a functional mammary gland basis. Lipid in the parenchymal tissue was not affected by dietary treatment (P>.88). When these results were compared per kg of maternal body weight (Table 5), total DNA, RNA, and protein were significantly reduced by elevated energy. Total lipid remained unaffected by treatment.

Concentrations of DNA, RNA, protein and lipid in the parenchymal and extraparenchymal stromal tissues are presented in Tables 6 and 7 respectively. Protein level did not effect, DNA, RNA or protein concentration of the parencymal tissue but tended to have a direct relationship with concentration of lipid (P<.09). Energy level did not affect the nucleic acid concentration of the parenchymal tissue but high energy tended to decrease protein concentration (P<.06) while increasing lipid concentration (P<.08; Table 6). Neither protein nor energy level affected the concentration of any of the constituents measured in the extraparenchymal stromal tissue (Table 7) nor did they affect the RNA to DNA ratios.

The effects of dietary treatment on total

extraparenchymal stromal nucleic acid on a functional gland basis is presented in Table 8. Neither dietary protein nor energy had any effect on the components measured. Results were not affected by equalizing for maternal body weight (Table 9).

Sow Weights and Carcass Characteristics

The effects of dietary protein and energy on sow weight and carcass composition are listed in Table 10. Gilts fed elevated levels of protein were lighter at d 75 than gilts fed adequate protein ($P<.05$). Differences were not apparent at the time of slaughter. Energy did not effect the weight of gilts at d 75. Gilts fed elevated energy gained more weight during the treatment period and were significantly heavier on d 105 of gestation.

Dietary protein level did not affect the full (weight including fetuses and fluid) or empty utero-placental weights, carcass weight, 10th rib backfat thickness or loin eye area of gilts. Dietary energy had no effect on full or empty utero-placental weights or loin eye area. However, increased energy levels tended to increase carcass weight ($P<.06$) and significantly increased 10th rib backfat thickness.

Fetal Characteristics

Protein and energy levels in the diets failed to affect the number of fetuses at d 105 of gestation.

Similarly, there was no effect of dietary treatment on fetal body weight or fetal liver weight at d 105 of gestation. Glycogen storage was not affected by dietary treatment since there was no difference in either concentration or total glycogen in the liver (Table 11).

TABLE 2. CHARACTERISTICS OF MAMMARY GLANDS OF GILTS AT TWO DIFFERENT STAGES OF GESTATION[a]

Trait	Feeding level			
	d 75	SD	d 105	SD
Number of gilts	3		32	
Avg wt, kg	133.2	5.0	153.4	10.4
Parenchymal wt, g	478.3	87.5	1011.6	274.5
Extraparenchymal stroma, g	410.4	109.9	686.7	158.0
DNA, mg/g	.7	.3	2.8	.6
Total DNA, g	.3	.2	2.9	.9
RNA, mg/g	.6	.1	3.5	.6
Total RNA, g	.3	.03	3.6	1.2
Protein, mg/g	27.4	5.8	14.6	29.3
Total protein, g	13.4	5.2	125.8	51.6
Lipid, mg/g	446.0	79.0	140.0	2.0
Total lipid, g	216.6	75.4	138.9	30.2

[a]No statistical analysis performed on these data.

TABLE 3. EFFECTS OF PROTEIN AND ENERGY ON MAMMARY PARENCHYMAL AND EXTRAPARENCHYMAL STROMA WEIGHTS[a]

Tissue	Feeding level E	Feeding level A	SE[b]	P[c]
	Protein			
Tissue wt/gland, g				
Parenchyma[d]	136.46	154.02	9.67	.21
Stroma[e]	91.20	105.82	5.91	.09
Gland wt/maternal BW[f], g				
Parenchyma	.95	1.05	.07	.28
Stroma	.63	.72	.04	.08
	Energy			
Tissue wt/gland, g				
Parenchyma	133.14	157.34	9.68	.08
Stroma	101.54	95.48	5.92	.47
Gland wt/maternal BW, g				
Parenchyma	.88	1.12	.07	.02
Stroma	.67	.68	.04	.92

[a]Based on right side mammary glands.
[b]Standard error of the mean.
[c]Probability level.
[d]Trimmed wt of parenchymal tissue.
[e]Weight of non-secretory tissue less skin, muscle and lymph nodes.
[f]Tissue wt/(gland·kg maternal body wt).

TABLE 4. EFFECTS OF PROTEIN AND ENERGY ON TOTAL NUCLEIC ACID, PROTEIN AND LIPID IN THE MAMMARY PARENCHYMAL TISSUE PER FUNCTIONAL MAMMARY GLAND.

Constituent	Feeding level E	Feeding level A	SE[a]	P[b]
	Protein			
DNA, mg	382.46	436.36	32.77	.25
RNA, mg	478.76	553.50	38.76	.18
Protein, g	18.49	19.15	2.05	.82
Lipid, g	19.41	18.93	1.09	.75
	Energy			
DNA, mg	367.34	451.48	32.77	.08
RNA, mg	458.00	574.28	38.80	.04
Protein, g	15.13	22.51	2.05	.02
Lipid, g	19.07	19.28	1.09	.89

[a]Standard error of the mean.
[b]Probability level.

TABLE 5. EFFETS OF PROTEIN AND ENERGY ON TOTAL NUCLEIC ACID, PROTEIN AND LIPID IN THE MAMMARY PARENCHYMAL TISSUE PER FUNCTIONAL GLAND EVALUATED PER KG MATERNAL BODY WEIGHT

Constituent	Feeding level E	A	SE[a]	p[b]
	Protein			
DNA, mg	2.71	2.99	.22	.31
RNA, mg	3.41	3.76	.28	.38
Protein, g	.13	.13	.01	.85
Lipid, g	.14	.14	.01	.78
	Energy			
DNA, mg	2.48	3.22	.22	.03
RNA, mg	3.09	4.07	.28	.02
Protein, g	.10	.16	.01	.01
Lipid, g	.13	.15	.01	.15

[a]Standard error of the mean.
[b]Probability level.

TABLE 6. EFFECTS OF PROTEIN AND ENERGY ON NUCLEIC ACID, PROTEIN AND LIPID CONCENTRATION OF MAMMARY PARENCHYMAL TISSUE

Constituent	Feeding level E	Feeding level A	SE[a]	P[b]
	Protein			
DNA, ug/g	2826.92	2840.47	158.44	.96
RNA, ug/g	3581.36	3538.51	150.32	.84
RNA/DNA	1.29	1.27	.05	.77
Protein, mg/g	133.93	118.27	7.78	.16
Lipid, mg/g	149.03	136.58	5.00	.09
	Energy			
DNA, ug/g	2765.39	2922.71	158.44	.49
RNA, ug/g	3476.47	3643.39	150.32	.44
RNA/DNA	1.28	1.28	.05	.95
Protein, mg/g	114.98	137.21	7.92	.06
Lipid, mg/g	149.71	135.91	5.00	.08

[a]Standard error of the mean.
[b]Probability level.

TABLE 7. EFFECTS OF PROTEIN AND ENERGY ON NUCLEIC ACID, PROTEIN AND LIPID CONCENTRATION IN MAMMARY EXTRAPARENCHYMAL STROMAL TISSUE

Constituent	Feeding level E	Feeding level A	SE[a]	p[b]
	Protein			
DNA, ug/g	118.06	106.70	8.64	.36
RNA, ug/g	360.89	341.77	26.91	.62
RNA/DNA	3.12	3.26	.19	.77
Protein, mg/g	30.15	28.61	2.04	.59
Lipid, mg/g	589.38	575.00	.19	.77
	Energy			
DNA, ug/g	115.61	109.16	8.64	.60
RNA, ug/g	353.93	348.73	26.92	.89
RNA/DNA	3.12	3.23	.19	.95
Protein, mg/g	27.06	31.70	2.04	.12
Lipid, mg/g	59.81	56.62	1.88	.24

[a]Standard error of the mean.
[b]Probability level.

TABLE 8. EFFECT OF PROTEIN AND ENERGY ON TOTAL NUCLEIC ACID, PROTEIN AND LIPID IN THE MAMMARY EXTRAPARENCHYMAL STROMAL TISSUE PER FUNCTIONAL MAMMARY GLAND

Constituent	Feeding level E	Feeding level A	SE[a]	P[b]
	Protein			
DNA, mg	10.65	11.43	1.11	.62
RNA, mg	32.60	36.50	3.40	.42
Protein, g	2.65	2.96	.18	.24
Lipid, g	54.15	61.20	4.09	.24
	Energy			
DNA, mg	11.48	10.60	1.12	.58
RNA, mg	33.23	35.85	3.40	.59
Protein, g	2.68	2.93	.18	.35
Lipid, g	60.52	54.82	4.09	.34

[a]Standard error of the mean.
[b]Probability level.

TABLE 9. EFFECTS OF PROTEIN AND ENERGY ON TOTAL NUCLEIC ACID, PROTEIN AND LIPID IN THE MAMMARY EXTRAPARENCHYMAL STROMAL TISSUE PER FUNCTIONAL GLAND EVALUATED PER KG MATERNAL BODY WEIGHT

Constituent	Feeding level E	Feeding level A	SE[a]	P[b]
	Protein			
DNA, mg	.07	.08	.22	.71
RNA, mg	.23	.23	.01	.48
Protein, g	.02	.02	.001	.34
Lipid, g	.37	.41	.03	.25
	Energy			
DNA, mg	.08	.07	.01	.91
RNA, mg	.24	.23	.02	.94
Protein, g	.02	.02	.001	.12
Lipid, g	.40	.39	.03	.74

[a]Standard error of the mean.
[b]Probability level.

TABLE 10. EFFECTS OF DIETARY PROTEIN AND ENERGY ON SOW WEIGHT AND CARCASS COMPOSITION

Trait	Feeding level E	Feeding level A	SE[a]	P[b]
	Protein			
d 75 weight, kg	133.43	140.45	2.31	.04
d 105 weight, kg	151.56	156.05	2.43	.20
Utero-placental full wt[c],kg	15.75	17.91	.99	.13
Utero-placental empty wt[d]kg	7.22	7.61	.46	.55
10^{th} rib fat, cm_2	2.16	2.39	.15	.25
Loin eye area,cm^2	36.38	35.09	1.15	.45
Carcass wt, kg	86.76	88.65	1.69	.44
	Energy			
d 75 weight, kg	134.34	139.54	2.31	.12
d 105 weight, kg	158.21	149.40	2.43	.02
Utero-placental full wt,kg	16.14	17.52	.99	.32
Utero-placental empty wt,kg	7.14	7.69	.46	.41
10^{th} rib fat, cm_2	2.64	1.89	.14	.001
Loin eye area,cm^2	36.38	35.09	1.15	.45
Carcass wt, kg	88.65	86.76	1.69	.05

[a]Standard error of the mean.
[b]Probability level.
[c]Wt including fetuses and fluid.
[d]Wt without fetuses and fluid.

TABLE 11. EFFECTS OF DIETARY PROTEIN AND ENERGY ON FETAL BODY WEIGHT AND GLYCOGEN STORAGE AT D 105 OF GESTATION

Trait	Feeding level E	Feeding level A	SE[a]	p[b]
	Protein			
Number of fetuses	8.37	9.19	.60	.34
Avg. fetus wt, g	946.32	969.28	42.53	.71
Total fetus wt, kg	8.78	7.62	.60	.15
Fetal liver wt, g	24.21	24.04	1.42	.93
Liver glycogen conc,[c] mg/g	63.14	66.91	5.83	.65
Total liver glycogen, g	1.52	1.53	.16	.94
	Energy			
Number of fetuses	8.25	9.31	.60	..22
Avg. fetus wt, g	946.26	969.34	42.53	.17
Total fetus wt, kg	7.64	8.77	.60	.70
Fetal liver wt, g	24.05	24.20	1.42	.94
Liver glycogen conc,[c] mg/g	60.35	69.70	5.83	.27
Total liver glycogen, g	1.57	1.50	.16	.65

[a]Standard error of the mean.
[b]Probability level.
[c]Mean concentration of glycogen from 5 fetuses per sow.

DISCUSSION

Mammary Development

The hypothesis of this experiment was that increased dietary protein and/or energy during late gestation would enhance mammary growth during pregnancy, as measured by DNA content. The results, however, demonstrate that the opposite may be true.

Parenchymal and extraparenchymal stroma weights were quite variable. Technique of dissection may affect the weights of these component tissues because there is no clear line of demarcation between them. Also, because the parenchymal tissue expands from an immature duct system into the stromal tissue (Tucker, 1987), different concentrations of intraparenchymal fat may also affect the weights of these tissues.

There was a tendency for decreased mammary parenchymal weight in gilts fed elevated energy when data was presented on a per gland basis. This difference became significant when the amount of parenchyma was adjusted for maternal body weight (weight of the sow less the weight of the fetuses). These results agree with previously reported data from prepubertal heifers (Sjersen et al., 1982) and lambs (Johnsson and Hart, 1985). However, Sjersen et al. (1982) observed a concomitant increase in the weight of the extraparenchymal

stromal tissue. This effect was not observed in the present study. Protein had no effect on parenchymal weight but gilts fed elevated protein tended to have less extraparenchymal stroma.

Data on the components of the extraparenchymal stromal tissue were in agreement with data previously reported by Sjersen (1981). It is not surprising that dietary protein and energy levels had no effect on the composition of extraparenchymal stroma, since the this tissue was comprised mainly of lipid. One might expect nutritional factors to affect the weight of the fatty tissue associated with the mammary gland, but not the composition of adipose gain.

Protein and energy did not affect nucleic acid concentration of the parenchymal tissue. These results are consistent with the results from prepubertal heifers fed high or low planes of nutrition (Sjersen et al., 1982). This would infer that diet does not affect composition of mammary development and therefore any differences in total DNA would be due to differences in total amounts of secretory tissue.

Protein concentration in the parenchyma of mammary glands was not affected by dietary protein level. In contrast, the lipid concentration in the gland tended to be increased by high dietary protein. Possibly, excess protein in the diet was converted to energy and deposited in the mammary gland as adipose tissue. This would suggest that the protein levels suggested by NRC (1979) are sufficient to meet the amino acid requirements of gilts for mammary growth.

Protein and lipid concentrations in the parenchymal portion of the glands tended to be affected by energy level fed ($P<.06$ and $P<.08$ respectively). Gilts fed adequate energy tended to have a higher concentration of protein and a lower concentration of lipid. Gilts fed elevated energy had more fatty tissue engulfed in the parenchymal tissue.

These results are contrary to the results of Park et al. (1987). He found that rats fed high protein and low energy, from the time they were weaned through lactation, had increased RNA and explants from these glands had greater protein secretion. Severe protein restriction on the other hand, has been associated with impaired mammary development as measured by total DNA (Singh and Turner, 1971). Our treatments in this experiment however, consisted of adequate and elevated protein levels. Our data suggests that excess protein is of no benefit and may, in fact, be a disadvantage to total DNA found in the mammary gland parenchyma.

Chew et al. (1984) reported that high protein levels had adverse affects on total DNA in prepubertal rats. In that study, rats fed to meet their protein requirement had greater total DNA than rats fed either high or low protein. These data support speculation that the effects of protein on mammary development are small unless diets become either severely restricted or are in great excess of the requirement.

Neither dietary energy nor protein level affected any of the stromal constituents measured. This is in agreement with the findings of Sjersen (1981) who found that plane of nutrition

had no effect on RNA, DNA, lipid or hydroxyprolene content of the stroma.

In contrast to the limited effects of elevated dietary protein, dietary energy during late gestation had a marked effect on development of mammary secretory tissue. Previous researchers found that increased energy during gestation increased gross mammary weight (Noblet et al., 1985) and the linear dimensions of the udder (Mellor and Murray, 1985; Mellor et al. 1987). The value of these experiments in relation to mammary development is limited, due to the fact that measurements of gross mammary weight and linear dimensions of the mammary gland do not measure amounts of secretory tissue present. It is conceivable that the increased dimensions and weights could be due to accumulation of fat in the mammary gland or may be reflective of an increase in total body weight of the animals studied.

In our experiment, elevated dietary energy levels had detrimental effects on total RNA and total protein of the parenchymal tissue. Elevated energy tended to reduce the total amount of DNA in the parenchyma when results were analyzed on a per functional gland basis. When expressed on a per gland basis and adjusted for maternal body weight, the detrimental effects of elevated energy during late gestation became very clear. There was no difference in total lipid but this can be explained by the fact that gilts fed elevated energy had less mammary parenchyma with a higher concentration of lipid, while the opposite was true for gilts fed low energy levels. This caused total lipid to be similar.

These results are similar to results obtained from prepubertal heifers (Sjersen et al., 1982) and sheep (Johnsson and Hart, 1985). Although the above studies were performed during the prepubertal growth period, the factors involved may be similar. Heifers (Sinha and Tucker, 1969a) and rats (Sinha and Tucker, 1969b) both undergo a prepubertal allometric growth phase just prior to puberty. The allometric growth phase seems to be related to the secretions of the ovary, since ovariectomy abolishes this allometric phase (Cowie, 1949). Also, this allometric growth phase coincides with the development of estrogen receptors in the mammary gland of mice. (Muldoon, 1979). Therefore, it has been postulated that ovarian secretions, primarily estrogens play a key role in stimulating this phase of growth.

Although a prepubertal allometric growth phase of the mammary gland has not been observed in swine, a similar phenomenon occurs in the development of porcine mammary glands during pregnancy. There is little change in the DNA content of the mammary gland up to d 50 of gestation (Hacker and Hill, 1972; Kensinger et al., 1982). However, at approximately d 70 of gestation, a tremendous proliferation of mammary tissue occurs. Maximal DNA contents occur at d 90 to 100 of pregnancy (Hacker and Hill, 1972; Kensinger et al., 1982).

It has been suggested that the allometric growth phase during pregnancy in swine is also signaled by estrogen secretion from the ovary and placental tissue (Kensinger et al., 1982; DeHoff et al., 1986). Plasma estradiol 17B

increases two-fold in the period from d 70 to d 105 of gestation (Robertson and King, 1974). Output of estrogens from the placenta increase 400-fold between d 70 and d 100 of gestation (Knight et al., 1977). This evidence suggests that the prepubertal allometric growth phases in other species may be similar to the allometric growth phase observed in pregnant swine. Thus, control of these events may also be similar and interactions between plane of nutrition and the hormones that control mammary growth may be comparable.

In ruminants, high dietary energy decreases serum GH levels (Sjersen et al., 1982; Johnsson et al., 1985). These decreases in GH concentrations have been associated with reduced DNA contents in the mammary gland suggesting a detrimental effect on mammary development. Injections of GH in prepubertal lambs fed ad libitum, restored DNA in the mammary gland to levels similar to that of animals on a restricted diet (Johnsson et al., 1986). Total parenchymal DNA was highly correlated (R=.95) to plasma growth hormone concentrations. From this information it seems that GH concentrations may have significant effects on the magnitude of the prepubertal growth phase in ruminants. During pregnancy, plasma levels of GH remain fairly constant in swine (DeHoff et al., 1986). But during fasting, GH levels in the pig increase with the length of fasting being indirectly related to blood glucose concentration (Machlin et al., 1967). Although GH is not thought to be mammiogenic in the pig during pregnancy (DeHoff et al., 1986), the effects of different plasma concentrations has not been established. It can

therefore, be speculated that diet induced differences in GH concentrations may occur, and may play a role in enhancing mammary development in pregnant gilts fed low levels of dietary energy.

Secretions from the ovary as mentioned above play a key role in development of functional secretory tissue. Energy restriction does not influence progesterone secretion in the pig (Hard and Anderson, 1979), even under starvation conditions. So it is not likely that dietary energy affects mammary development through effects on progesterone. Estrogen levels are affected only after inanition for long periods of time (greater than 30 d; Anderson, L.L., 1975). Therefore, secretions from the ovary are not easily affected by the nutrition of the sow during pregnancy.

Another hormone which is affected by nutrition, and plays a significant role in mammary development is prolactin. Prolactin is usually thought to be a lactogenic hormone. Prolactin concentrations in the pig remain constant during pregnancy until the periparturient period (DeHoff et al., 1986). Prolactin concentrations are reduced in obese mice (Sinha et al., 1975). Increased serum prolactin has been associated with periods of increased mammary development in rats (Sinha and Tucker, 1969b). Starvation has been shown to decrease serum prolactin (Campbell, 1977). Depressed DNA in the mammary gland of the prepubertal ewes fed a high plane of nutrition was associated with increased prolactin concentrations (Johnsson et al., 1985). Thus, it seems

unlikely that energy's effect on development of the mammary gland is due to a nutritional effect on prolactin.

Insulin is also affected by nutrition. Yet, Johnsson et al. (1985) observed no difference in plasma response to restricted feeding in sheep. Insulin's role in mammary development is one of permission (Tucker, 1981) and the literature to date does not support the concept that plasma concentrations above the concentration required to support mammary development has any affect.

In this discussion, only blood concentrations of selected mammiogenic and lactogenic hormones have been discussed. Other factors may affect the biological response to these hormones including the number of receptors and synergism between hormones. The interaction between nutrition and the endocrine system is not fully understood. Further knowledge in this area may alter the implications of our data.

The Sow and Conceptus

The effects of protein and energy on the number of fetuses were not surprising. The results are supported by several researchers who have found no effect of protein (Holden et al., 1968; Baker et al., 1970; Shields et al., 1980) or energy (Lodge, 1966; Baker et al., 1969; Whittemore et al., 1984) on the number of pigs born. Likewise, fetal weight was not affected by dietary protein. This is in agreement with other researchers who have found no effect of protein level during pregnancy on birth weight (NCR-42 Committee on Swine Nutrition Research, 1978) when reasonable protein levels were fed. Only under severe protein

restriction has protein had an effect on birth weight (Pond, 1968).

However, energy affects fetal weights at d 112 of gestation (Noblet et al., 1985) and birthweight (Cromwell et al., 1982). There was no effect of energy on birthweight in this study. This may be due to the fact that fetuses were sacrificed prior to the period of maximal fetal growth (Noblet et al., 1985).

Fetal liver glycogen storage was not affected by protein or energy level fed. This is in agreement with previous reports by Boyd et al. (1978) and Okai et al. (1978). However, pigs in our study were sacrificed before maximal glycogen storage occurred (Mersmann, 1974).

Protein had no effect on the weight of sows at d 105. Sows fed elevated energy during gestation were significantly heavier at d 105 than sows fed adequate energy. This effect of energy on gestation weight gain has been well documented (Verstagen, 1987). In addition, gilts fed high energy had more backfat than gilts fed adequate energy. The increase in fat in the carcass was probably responsible for the significant increase in carcass weight observed in gilts fed elevated energy.

SUMMARY

In conclusion, the NRC (1979) requirements for pregnant gilts are sufficient to allow normal development and the development of the products of conceptus. High levels of dietary energy, but not high protein during the period of maximal mammary development in swine (d 70 to d 105 of gestation) may have deleterious effects on the development of mammary glands and may impair subsequent lactational performance. The effects of high energy during lactogenesis (d 105 to d 112) on mammary development have yet to be elucidated.

The effects of diet on the development of the mammary gland, especially during pregnancy, is an area where little data exists. More data needs to be collected so that we can better understand the mechanisms by which nutrition interacts with mammary function.

APPENDIX

APPENDIX 1 MAMMARY DATA

gilt	b	p	e	mwt	pcm	str	g	f	wt	p	p DNAc	pRNAc	pPROc
106-1	1	2	1	6683.00	1620.80	691.30	7	9	342	3	2552.27	3854.12	151.32
106-2	1	1	2	5898.00	977.00	772.30	7	8	341	3	2150.45	3350.42	120.70
107-3	1	1	2	5534.00	955.30	625.60	8	4	317	3	3465.08	4097.74	187.50
108-3	1	1	1	4315.00	790.10	591.90	7	11	364	3	2846.73	3222.00	136.78
112-5	1	2	1	5036.50	812.80	824.10	7	10	342	1	3540.47	3783.58	164.70
113-1	1	1	2	5397.00	865.80	825.30	7	5	318	3	2267.76	3249.89	92.41
114-3	1	2	2	3579.00	702.80	456.80	7	9	316	3	2618.94	3748.97	87.11
139-6	1	2	1	5183.00	1277.70	514.30	7	10	329	2	2884.31	2725.69	206.10
206-1	1	2	2	4263.00	909.00	516.40	8	9	324	3	1918.09	2938.87	91.86
215-1	1	1	1	5896.00	1236.70	748.50	7	10	352	1	2681.84	4518.53	101.60
215-3	1	1	2	5773.00	845.00	901.30	7	9	364	1	2649.05	3091.53	113.28
215-5	1	2	2	6628.20	1049.30	1102.20	7	6	365	1	2717.42	3863.09	114.27
218-3	1	1	2	5461.00	1017.80	815.10	7	8	383	1	2713.36	3685.09	85.30
219-1	1	2	2	5080.00	789.20	840.90	7	10	392	1	2426.74	3329.52	119.35
219-4	1	1	1	6299.00	1576.00	814.80	6	10	334	1	3096.13	3935.06	136.21
22-5	2	1	1	4928.60	712.50	686.10	6	9	331	2	3959.17	3417.05	116.65
23-2	2	1	2	5550.00	1061.00	831.00	7	9	352	2	4622.85	3019.86	118.60
23-4	2	2	1	3976.00	752.10	510.00	7	9	314	2	3029.00	3669.89	93.14
245-6	1	2	2	4824.00	869.60	561.50	6	3	334	2	3392.05	3657.50	115.62
24-3	2	2	2	5641.00	955.50	695.70	7	5	337	2	3394.64	3404.81	155.95
250-1	1	1	2	4625.00	969.20	685.90	8	6	326	2	2133.62	2751.39	117.57
250-2	1	1	1	7250.00	1639.50	890.80	7	8	329	2	2233.59	3459.45	115.16
253-3	1	1	1	5498.00	962.50	772.40	7	10	351	2	3005.52	4191.77	100.98
25-2	2	2	1	4978.00	835.70	620.10	6	10	323	2	2422.31	4093.67	138.68
27-1	2	2	1	4535.00	714.80	592.00	7	6	318	2	1794.84	2021.17	72.73
27-4	2	2	2	5517.00	1150.10	676.90	8	10	344	2	3868.42	4474.52	126.83
27-5	2	1	1	4487.00	1010.00	378.80	7	12	298	2	3381.85	3782.98	105.35
2-3	2	2	1	4103.00	870.30	438.40	7	12	287	3	3294.47	4745.30	158.09
35-1	2	1	1	7215.00	1662.00	822.00	8	9	328	3	2064.37	2540.39	900.00
35-4	2	1	2	5540.00	1179.00	692.90	7	12	376	3	2469.95	3704.50	138.50
38-5	2	1	2	3868.00	693.50	561.20	7	11	346	3	2537.96	3220.58	101.61
4-4	2	2	2	5440.00	909.00	516.40	8	12	351	3	2485.72	3366.91	101.34

KEY TO HEADINGS:

b=breed
p=protein
e=energy
pcm=parenchema
str=stroma
g=glands
f=fetuses
wt=weight of sow,lbs
p (second)=period
pDNAc=pcm DNA concentration
pRNAc=pcm RNA concentration
pPROc=pcm protein concentration

APPENDIX 2. MAMMARY GLAND DATA (cont.)

gilt	pLIPc	sDNAc	sRNAc	sPROc	sLIPc	pDNAt	pRNAt	pPROt	pLIPt	pLIPt
106-1	0.13	97.18	292.09	22.97	0.60	4136.71	6246.76	245.25	207.22	67.18
106-2	0.14	80.52	366.68	23.32	0.59	2100.99	3273.36	117.92	139.25	62.19
107-3	0.15	71.90	193.10	19.52	0.55	3310.19	3914.57	179.12	142.34	44.98
108-3	0.13	91.92	345.56	31.39	0.57	2249.20	2545.71	108.07	105.24	54.41
112-5	0.16	105.57	429.81	32.76	0.59	2877.70	3075.30	133.87	131.98	87.00
113-1	0.15	112.64	412.34	35.79	0.46	1963.43	2813.76	80.01	127.10	92.96
114-3	0.13	132.98	352.75	37.10	0.67	1840.59	2634.77	61.22	92.42	60.75
139-6	0.10	137.54	485.17	63.55	0.55	3685.28	3482.62	263.33	123.07	70.73
206-1	0.16	173.55	468.56	40.55	0.55	1743.54	2671.44	83.50	143.84	89.62
215-1	0.12	154.50	512.77	25.01	0.54	3316.64	5588.07	125.65	149.48	115.64
215-3	0.15	111.40	312.22	31.21	0.65	2238.45	2612.34	95.72	128.24	100.40
215-5	0.12	141.47	398.19	22.79	0.54	2851.39	4053.54	119.90	121.49	155.92
218-3	0.13	165.42	496.52	24.78	0.52	2761.66	3750.68	86.81	132.24	134.83
219-1	0.17	54.15	218.78	17.12	0.67	1915.18	2627.66	94.19	134.14	45.53
219-4	0.12	111.53	280.83	28.88	0.51	4879.50	6201.65	214.66	193.75	90.87
22-5	0.13	120.71	412.50	33.28	0.60	2820.91	2434.65	83.11	91.73	82.82
23-2	0.13	77.52	415.65	23.60	0.71	4904.84	3204.07	125.83	136.76	64.42
23-4	0.16	141.31	495.37	33.84	0.51	2278.11	2760.12	70.05	118.56	72.07
245-6	0.12	134.72	501.53	0.00	0.59	2949.72	3180.56	100.54	108.12	75.64
24-3	0.20	62.49	146.03	20.46	0.62	3243.57	3253.29	149.01	190.68	43.47
250-1	0.17	108.83	327.35	24.07	0.59	2067.90	2666.64	113.95	161.95	74.65
250-2	0.50	131.64	338.69	28.30	0.60	3661.96	5671.77	188.81	262.32	117.27
253-3	0.12	64.32	190.69	27.85	0.62	2892.82	4034.58	97.19	117.55	49.68
25-2	0.16	168.34	377.65	37.67	0.76	2024.32	3421.08	115.89	129.61	104.39
27-1	0.18	92.43	349.39	25.49	0.64	1282.95	1444.73	51.98	126.77	54.72
27-4	0.15	137.25	303.37	32.42	0.59	4449.07	5146.14	145.87	173.66	92.91
27-5	0.13	82.28	303.69	56.22	0.41	3415.66	3820.81	106.40	131.01	31.17
2-3	0.14	79.82	297.82	43.62	0.48	2867.17	4129.83	137.58	121.74	34.99
35-1	0.81	95.61	274.62	26.21	0.53	3430.98	4222.13	295.87	199.44	78.59
35-4	0.12	72.24	157.09	18.94	0.60	2912.07	4367.61	163.29	147.00	50.06
38-5	0.15	126.18	321.10	18.87	0.70	1760.07	2233.48	70.47	101.32	70.81
4-4	0.15	158.33	464.65	31.29	0.52	2259.52	3060.52	92.12	136.53	81.76

KEY TO HEADINGS:

pLIPc=pcm lipid concentration
sDNAc=str DNA concentration
sRNAc=str RNA concentration
sPROc=str protein concentration
sLIPc=str lipid concentration
pDNAt=pcm DNA total
pRNAt=pcm RNA total
pPROt=pcm protein total
pLIPt=pcm lipid total

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