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THE EFFECT OF A PRE-PUBERTAL DIETARY RESTRICTION ON SERUM GROWTH HORMONE CONCENTRATION AND FUTURE MILK PRODUCTION OF REPLACEMENT EWES

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By

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ABSTRACT

THE EFFECT OF A PRE-PUBERTAL DIETARY RESTRICTION ON SERUM GROWTH HORMONE CONCENTRATION AND FUTURE MILK PRODUCTION OF REPLACEMENT EWES

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Traditionally, sheep producers have chosen their heaviest ewe lambs as replacement females. Increasing plane of nutrition in pre-pubertal heifers and ewes may reduce serum growth hormone level and subsequently impair the development of mammary secretory tissue, reducing milk production. Measuring mammary development is a subjective, often terminal process, making it difficult to correlate mammary secretory tissue with future milk production.

Restricting pre-pubertal dietary intake in ewes increased serum growth hormone concentration but did not affect first lactation milk production, however, Restricted ewes produced more milk as a percent of body weight and milk of higher percent fat than ad libitum fed Control ewes. There was no difference in lamb gain between lambs reared by Control and Restricted ewes. Computed Tomography (CT) provided a method by which to evaluate mammary development and composition in the live ewe.

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Chapter One

INTRODUCTION

Traditionally, sheep producers have chosen their largest, heaviest ewe lambs as replacement females. By using ewe size and weight as selection criteria, producers may compromise future milk production, lamb gain and ultimately, profit. An increased pre-pubertal plane of nutrition in dairy heifers and ewe lambs may reduce milk production of those females in future lactations by inhibiting mammary development (Sejrsen, 1981; Johnnson and Obst, 1984). The number of milk secreting cells present in the mammary gland limits milk production (Tucker, 1969). Although a high plane of nutrition in heifers and ewe lambs prior to puberty may reduce future milk production, the same does not appear to be true of a high plane of nutrition after puberty (LaCasse et. al.; 1993, Johnnson and Obst, 1984).

While a high pre-pubertal plane of nutrition may be detrimental to future milk production, simply restricting dietary intake in the interest of improving future milk production may also be detrimental. An animal consuming enough energy to support the maintenance of body function with little energy left over for growth, will experience a reduced rate of gain. McCann et al. (1989) determined that ewes gaining 239 $g \cdot d^{-1}$ from weaning to the onset of puberty reached puberty at a younger age than ewes gaining 179 $g \cdot d^{-1}$ during the same period. This delay in reproductive ability could be economically detrimental if a

producer wishes to breed ewe lambs. Compensatory growth feeding strategies may provide alternatives to simply reducing energy intake during pre-pubertal development in the interest of improving future milk production without sacrificing over-all growth.

By definition, animals fed for compensatory growth are on a restricted plane of nutrition for a period of time and are subsequently switched to a higher plane of nutrition for an additional period of time. In general, animals on a compensatory growth feeding regimen exhibit greater efficiency of gain (Ellenberger et al., 1989) and an altered endocrine status when compared to ad libitum fed controls (Wester et al., 1995; Ellenberger, 1989). Five-month-old lambs restricted in dietary energy or protein for a 7 week period exhibited decreased IGF-I and increased growth hormone in serum. A 2 week repletion of dietary energy or protein resulted in a drop in serum growth hormone levels to that of ad libitum fed controls and IGF-I increased above control levels (Wester et al., 1995). Similarly, beef cattle fed restricted intakes to gain .37 kg/d from 240 to 307 kg exhibited elevated serum growth hormone (GH) and decreased IGF-I levels as compared to ad libitum fed control steers gaining 1.4 kg/d. Upon realimentation, serum IGF-I levels in restricted cattle increased above those of controls, while serum GH returned to control levels (Ellenberger et al., 1989). Sejrsen and coworkers (1983) suggested that the relationship between a diminished plane of nutrition and increased mammary parenchymal cell number

may be due to the increase of serum growth hormone and prolactin, during the prepubertal, allometric growth phase in the mammary gland. Feed restriction followed by realimentation in a stair-step fashion resulted in a greater number of mammary parenchymal cells, reduced fat and increased milk production in dairy heifers (Park et. al, 1989). By implementing a compensatory growth feeding regimen with pre-pubertal restriction followed by realimentation, it may be possible to improve future milk production without impairing growth rate or the onset of puberty.

Studying mammary development or composition as it relates to milk production is a complicated process. To obtain mammary development and composition data, studies to date have relied primarily on udder dissection and chemical analysis (Swanson and Poffenbarger, 1979; Sejrsen, 1981; McCann et. al.; 1989, Johnsson and Hart, 1985). Due to the terminal nature of the method, milk production data from animals whose mammary glands are removed cannot be obtained or correlated to mammary composition. A non-terminal method of quantifying mammary parenchymal cells is needed to obtain correlations between mammary composition and future milk yield and to evaluate mammary development without sacrificing animals. Work by Glaser et al., (1991) and Sorensen et al., (1987) suggested that computer tomography (CT) may offer such a method.

A relationship exists between pre-pubertal plane of nutrition and future

milk production in ruminants. Animals on a high plane of nutrition prior to puberty tend to produce less milk than those on a lower plane of nutrition during the same period. Much of this work has studied the development of dairy and beef replacement females. The primary objective of the current study was to determine the effect of a compensatory growth feeding regimen on the future milk production of the replacement ewe. More specific objectives were to: 1) determine the effect of pre-pubertal dietary restriction followed by realimentation on growth and subsequent milk production of ewe lambs as compared to lambs reared on ad libitum intake diets; 2) compare growth hormone profiles of pre-pubertal ewes on restricted or ad libitum intakes; and 3) to evaluate the effectiveness of computer tomography as a means of quantifying mammary volume and composition in the live ewe.

Chapter Two

REVIEW OF LITERATURE

Mammary Growth and Development

Mammary Physiology

The function of the mammary gland is secretion of milk for the nourishment of offspring (Schmidt, 1971). The majority of existing mammals belong to the class Mammalia and sub-class Eutheria, members of which give birth to live young and have well developed mammary glands. These mammary glands consist of secretory tissue drained by a duct system and a teat. The size, shape and number of mammary glands differs across species, however, the histology and cytology of the secretory tissue is relatively similar from species to species (Schmidt, 1971).

The mammary gland consists of a system of branching ducts, with terminal ducts enlarging to form alveoli (Turner, 1952). The alveoli and terminal ducts are lined with a single layer of epithelial cells that synthesize and secrete milk. There is a high correlation (r^2=.50 to .85) between mammary epithelial cell number and milk yield, suggesting that increased mammary epithelial cell number results in increased milk yield (Tucker, 1969).

A group of alveoli is surrounded by connective tissue to form a distinct entity or lobule. The terminal ducts of each alveolus in a lobule join to form intralobular ducts which empty milk into an intralobular collection area. This intralobular collection space narrows and passes through the connective tissue as an interlobular duct. Similarly, a group of lobules is held together by connective tissue to form a lobe and the interlobular ducts of each lobule empty into an interlobular milk collection space, which again narrows and passes through the connective tissue of the lobe as an interlobar duct. The term parenchyma is used to describe the series of alveoli, lobules, lobes and ducts that comprise the epithelial component of the mammary gland. The connective tissue that separates and supports the parenchymal tissue is called stroma (Turner, 1952).

The Mammary Anatomy of the Ewe

The mammary gland anatomy of the ewe has been described by Turner (1952) and Schmidt (1971). The udder of the ewe consists of 2 mammary glands each drained by a single teat, and each teat is emptied by one streak canal. The gland cistern collects milk from the milk ducts, which carry it from the alveoli, lobules, lobes and ducts. The gland cistern then empties into the streak canal and milk exits the udder in this manner. Compared to other species, the gland cistern of the ewe is fairly small and irregular in shape (Turner, 1952).

The mammary gland of the ewe is supplied with blood by the external pudendal artery, which becomes the mammary artery after passing through the inguinal canal. Similar to the mammary artery of the cow, the vessel passes through the mammary tissue for a short time and then inclines cranially. Unlike the cow, however, the mammary arteries and veins do not branch into cranial and caudal mammary arteries and veins as they enter the udder. Instead, the external pudendal arteries and veins enter the mammary gland close to it's posterior border. As the mammary artery passes cranially it extends a deep medial branch, which continues anteriorly, extending deep medial branches. The mammary artery then terminates in the anterior basal border of the udder (Schmidt, 1971)

Blood leaves the mammary gland of the ewe by way of one of two veins; the external pudendal vein and the mammary vein. The external pudendal vein exits the udder and runs parallel to the external pudendal artery through the inguinal canal to the posterior vena cava. Entering the udder at the posterior basal border, the external pudendal vein turns anteriorly, sending branches into the udder parallel to those formed by the external pudendal artery. The mammary vein runs cranially, exiting the udder at it's anterior basal border and becomes the subcutaneous abdominal vein, which enters the thoracic cavity just behind the sternum.

Lymphatic vessels from each mammary gland of the ewe pass to the

supramammary lymph gland from both sides of the udder. At this point, a single lymphatic vessel enters the abdomen by way of the inguinal canal and runs parallel and caudal to the external pudendal blood vessels.

The udder of the ewe is supplied with inguinal nerves which separate into two branches. The superficial branch innervates the abdominal wall muscles, whereas the deep branch enters the inguinal canal and follows the external pudendal vessels to the udder. Once in the udder, the deep branch of the inguinal nerve again separates, sending two branches to innervate the arterial walls, milk ducts and teats (Schmidt, 1971).

Post-Fetal Growth and Development of the Mammary Gland

Birth to Puberty

At birth, the appearance of the udder is similar to that of an adult animal, though smaller in size (Schmidt, 1971). The vascular and lymphatic systems of the mammary gland are comparable to those found in the mature udder, as are the adipose and connective tissues (Schmidt, 1971).

Mammary parenchyma is immature at birth but exhibits allometric growth prior to puberty (Tucker, 1969). Most of the growth of the mammary gland from birth to puberty is due to an increase in connective and adipose tissue, however, some growth of parenchymal tissue also occurs during this period (Schmidt, 1971). The mammary area of the rat increased 1.13, 3.92 and .59 times faster than body surface area from 10 to 20, 23 to 40, and 60 to 100 days of age

respectively (Sinha and Tucker, 1966). Rats showed external signs of puberty at the 34-35th day of age, thus allometric growth occurred prior to or during the onset of puberty (Sinha and Tucker, 1966). Using changes in mammary DNA content as an indicator of cell proliferation, Sinha and Tucker (1969) found that mammary DNA increased 1.6 times faster than body weight from birth to 2 months of age in Holstein heifers. From 5 to 9 months of age, mammary DNA increased at 3.5 times the rate of body weight, subsequently falling to a growth rate 1.5 times that of body weight from 9 to 12 months of age. In the same study, age at first estrus ranged from 5.0 to 11.1 months with an average age of 7.4 months, thus, the most pronounced period of allometric mammary growth in these dairy heifers was just prior to or concurrent with the onset of puberty (Sinha and Tucker, 1969). Anderson (1975), reported that mammary growth in Romney and Romney-cross sheep is slow from birth to 3 months of age and rapid during the 4th month. Anderson found that a mammary growth plateau is reached at 5 months of age, the approximate age of puberty in the ewe, with little mammary growth during the next few months. Based on Anderson's data, the body weight of these ewes increased three-fold from 5.5 kg at birth to 18.9 kg at3 months, and the untrimmed mammary gland weight increased more than eight-fold, from 11 g at birth to 97 g at 3 months of age, suggesting that while mammary growth was slow in these pre-pubertal ewes, growth rate was allometric prior to puberty. Given the data reported by Sinha and Tucker (1966,

1969) and Anderson (1975) there is a period of allometric mammary growth prior to or concurrent with puberty in the rat, heifer and ewe.

Post-Puberty through Lactation

The allometric growth rate of the mammary gland of the rat, heifer and the ewe continues for several estrous cycles and then returns to an isometric growth rate (Sinha and Tucker, 1966, Sinha and Tucker, 1969, Anderson, 1975). During the phase between puberty and conception, mammary ducts branch and re-branch with each recurring estrous cycle (Schmidt, 1971). Using total mammary gland nucleic acid content as an index of cell proliferation in rats, Sinha and Tucker (1966) reported an increase in deoxyribonucleic acid (DNA) content per 100 g of body weight during the first four estrous cycles, with no further increase after the fourth cycle. As in rats, Holstein heifers exhibited increased mammary DNA during the first few estrous cycles after which a plateau was reached until pregnancy occurred (Sinha and Tucker, 1969). Anderson (1975) reported that a mammary growth plateau was reached at 5 months of age, the approximate age of puberty in the ewe, with little change observed during the next few months.

The majority of mammary growth occurs during pregnancy (Schmidt, 1971). Further extension and branching of mammary ducts occurs during pregnancy (Cowie, 1971), and functional alveoli develop to replace lipid in the mammary fat pad (Tucker, 1969). In dairy cattle, development of the mammary

gland is continuous and exponential throughout gestation, with parenchymal weight increasing at a rate of 25% per month (Swanson and Poffenbarger, 1979). Anderson (1975) reported an increase in total mammary DNA from 438 mg at the start of pregnancy to 2,330 mg near the end of pregnancy in Romney and Romney-cross ewes. Seventy-eight percent of mammary development in the ewe occurs during pregnancy, with 20% occurring from birth to puberty and 2% occuring during fetal development (Anderson, 1975).

Mammary cell numbers increase during early lactation in goats and dairy cattle (Knight and Peaker (1982) in Tucker, 1987 and Akers et al. (1981) in Tucker, 1987), but not in sheep (Anderson, 1975). There was no significant change in the mammary DNA of Romney and Romney-cross ewes between the end of pregnancy ; 2230 mg and5 days of lactation ; 2269 mg (Anderson, 1975).

In summary, mammary development proceeds at an isometric rate from birth until the onset of puberty in the rat, heifer and ewe. Prior to or concurrent with the onset of puberty, the mammary gland develops an allometric rate for several estrous cycles, subsequently returning to a post-pubertal , isometric rate of development until conception or maturity. The majority of mammary development occurs during pregnancy in the rat , heifer and ewe, continuing into the early stages of lactation in the rat and heifer, but not the ewe.

Endocrine Aspects of Mammary Development

Studies of the rat, mouse and goat have shown that a functional anterior

pituitary gland is required for the mammary gland to respond to ovarian hormones (Cowie, 1971). A series of experiments by Lyons and co-workers (Lyons 1958 in Sejrsen, 1981) showed that optimal duct growth in rats required growth hormone (GH), estrogen and glucocorticoids, while alveolar development required estrogen, progesterone, growth hormone, prolactin and glucocorticoids . While it is possible to stimulate udder development in ovarectomized dairy heifers using a combination of estrogen and progesterone, anterior pituitary hormones are also needed to optimize mammary growth (Tucker, 1969).

Sejrsen (1981) found that plane of nutrition effected mammary development during the pre-pubertal, allometric growth phase. In addition, he determined that changes in mammary development were correlated with concentrations of serum growth hormone. Eleven pre- and 11 post-pubertal Holstein-Friesian heifers were used to investigate the effect of plane of nutrition on mammary development and to determine if alterations in mammary growth were related to serum concentrations of mammogenic hormones. Cattle were assigned to treatment groups of ad libitum or restricted intakes. Dry matter intakes of the restricted heifers were 55% and 62% of heifers fed at ad libitum levels of intake, for pre and post-pubertal heifers, respectively. During the pre-pubertal period, heifers on ad libitum intakes were found to have a 23-40% decrease in amount and percent of mammary parenchyma, mammary DNA and percent epithelial cells. Body growth rate was negatively correlated with the

amount of parenchyma, percent parenchyma, mammary DNA and percent epithelial cells, but no relationship was shown between plane of nutrition and mammary growth in the post-pubertal period. Pre-pubertal heifers on restricted intakes had elevated serum GH, which correlated positively with mammary parenchyma (r^2 = .55, P<.10), percent parenchyma (r^2 =.75, P<.01), mammary DNA (r^2 =.54, P<.10) and percent epithelial cells (r^2 = .48, P<.15). Plane of nutrition had no effect on serum GH concentration during the post-pubertal phase.

Johnsson et al., (1985) conducted a similar study to examine the effect of level of nutrition on plasma concentration of GH, insulin and prolactin at various ages in ewe lambs, and the relationship between those concentrations and mammary development. Crossbred ewe lambs were randomly assigned to a high (H) or low (L) dietary treatment from 4 to 20 and (or) 20 to 36 weeks of age. Diets consisted of a 95:5 concentrate: forage ration. High-group lambs were fed to gain 220 $g \cdot d^{-1}$ and L lambs were fed to gain 110 $g \cdot d^{-1}$. Blood samples were collected from five lambs on each treatment at 1-or-2 hour intervals over a 28-hour period at 10, 14, 18, 26 and 34 weeks of age. Serum samples were analyzed for serum GH, prolactin and insulin and lambs were slaughtered at 20 or 36 weeks of age for determination of mammary development . Johnsson et al. (1985) reported that mean plasma GH and the effect of level of nutrition on mean plasma GH declined as age increased. In addition, the greater mammary

development of L lambs as measured by parenchymal dried, fat-free tissue (DFFT) and parenchymal DNA was associated with increased concentrations of GH in plasma (Johnsson et al., 1985).

Johnsson et al. (1985) and Sejrsen (1981) provide evidence that in pre-pubertal sheep and cattle, a decreased plane of nutrition increases concentration of GH in serum (Sejrsen, 1981) and plasma (Johnsson et al. 1985). In turn, a positive correlation exists between greater concentration of GH in serum or plasma and amount of parenchymal tissue in the mammary gland (Sejrsen, 1981; Johnsson et al., 1985). The mechanism by which GH increases total mammary parenchyma is not clear, in that GH receptors have not been located in mammary parenchyma (McFadden et al., 1990). Growth hormone effects on the mammary gland appear to be mediated indirectly through production of IGF-I from the liver (Akers, 1985).

Measuring Mammary Composition

Mammary gland composition in cattle, sheep and goats is often measured using dissection and chemical analysis (McCann et al., 1989; McFadden et al., 1990; Bowden et al., 1995). Mammary components that may be quantified in the dissection and chemical analysis procedure include: wet untrimmed and trimmed mammary weight, (DFFT) and protein and mammary DNA and RNA. In some studies, parenchyma and stroma are separated to calculate parenchymal

weight, DFFT, protein, DNA and RNA (Johnsson and Hart, 1985; Anderson, 1975). Anderson (1975) used dissection and chemical analysis to determine the mammary composition of Romney and Romney-cross ewes at seven stages of development including: fetuses at 140 to 148 days of gestation or lambs 5 days old, 3, 4 and 5 month-old lambs, 2 or 3 days of pregnancy, 140-148 days of pregnancy and 5 days of lactation. Johnsson and Hart (1985) determined the mammary composition of Hampshire Down X (Bluefaced Leicester X Swaledale) cross-bred ewes in a study examining the effects of level of nutrition on growth and pre-pubertal mammogenesis in sheep. Ewes were fed a concentrate pellet and forage ration to gain 220 $g \cdot d^{-1}$ (H) or 110 $g \cdot d^{-1}$ (L) from 1 to 5 and 5 to 8 months of age. Mammary composition data collected at 5 months of age in both the Anderson (1975) and Johnsson and Hart (1985) studies are summarized in Tables 1 and 2. Dissection of mammary parenchymal and extra-parenchymal tissue is difficult, since parenchymal tissue consists of ducts which branch into extra-parenchymal tissue. The parenchymal tissue is generally identified visually and separated from the extra-parenchymal tissue, which results in a highly variable, subjective process. This is evident in the range of values produced in the Anderson (1975) and Johnsson and Hart (1985) studies (Tables 1 and 2). Ewes in both studies are of similar body weight and age; however,

Table 1: Mammary Composition of 5-month-old Romney Ewes

Ewe breed	Body wt. (kg)	Age (mo)	Untrimmed mammary wet weight (g)	Trimmed mammary wet weight (g)	Total DFFT (g)	Protein (g)	DNA (mg)	RNA (mg)
Romney	29.9	5	113	78	4.6	2.89	92	105

(Adapted from Anderson, 1975)

Table 2: Mammary Composition of Five-month-old Crossbred Ewes

Treatment	Body wt. (kg)	Age (mos)	Trimmed mammary fat pad (g)	Fat pad containing parenchyma (g)	Parenchyma DFFT (mg)	Total DNA (mg)	Total RNA (mg)
High	33.2	5	29.99	8.21	623	25.9	23.5
Low	23.7	5	14.74	9.57	844	32.3	28.5

(adapted from Johnson and Hart, 1985)

Anderson reports total mammary DNA and RNA values of 92 and 105 mg, respectively, as compared to values of 26 and 24 mg as reported by Johnsson and Hart (1985). In addition to the variability of results produced by dissection and chemical analysis, animals must be slaughtered for mammary dissection and consequently, it is impossible to obtain milk production data from an animal whose mammary composition is known.

In an effort to eliminate the subjective, terminal nature of mammary gland dissection and analysis, researchers have begun to look at other methods of determining mammary gland composition. Stelwagen and Grieve (1990) conducted a morphometric evaluation of mammary composition obtained through biopsies of tissue from each quarter of the mammary gland in dairy cattle, however, the results correlated poorly to chemical analysis of dissected glands and were not considered useful. Stelwagen and Grieve (1990) postulated that biopsy may be of value in quantifying the mammary tissue of mature animals, which they suggest have more homogeneous tissue, as opposed to the developing heifers used in their study. Niezen et al. (1996) assessed the reliability of ultra-sound to measure mammary parenchymal tissue mass, determining that ultrasonic measurements at the base of the teat showed low, non-significant correlations with measurements from other quarters or with mass of DFFT. Correlation coefficients between ultrasonic area measurements

of the right and left halves of the mammary gland and the amount of right gland DFFT in post-pubertal Holstein heifers as determined by dissection and chemical analysis were -.081 and -.250 for the right and left glands, respectively (Niezen, 1996).

Computed tomography (CT) is a non-terminal method that may allow successful measurement of mammary composition in vivo. According to Seeram (1994), CT involves image reconstruction from emission or transmission measurements collected from the patient. This information is processed by a computer that uses mathematical techniques to build up sectional images of internal anatomy and the tissues therein. Computed tomography reports attenuation values for each sectional image due to differences in tissue density and the degree of attenuation is expressed in Houndsfield units (HU). Attenuation values of water, air and bone are 0, -1,000 and 1,000 HU , respectively (Seeram, 1994).

According to Sorensen et al. (1987) CT provided estimates of parenchyma more highly correlated to amount of fat-free parenchyma and parenchymal protein than to total parenchymal weight, as determined by dissection and chemical analysis. Mammary parenchymal weight and volume of twenty-five, 260 kg dairy heifers were quantified using CT, and udder dissection and chemical analysis (Sorensen et al., 1987). Mammary glands were

separated from the abdominal wall at slaughter and maintained at -18° C until scanning. Following CT scanning, glands were cut into 2 cm slices and slices dissected into extra-parenchymal and parenchymal tissue. Representative samples of total parenchyma were analyzed for dry matter, protein and fat content. Volumes of each tissue type were calculated from the areas obtained from CT scans taken 2 cm apart from the anterior to posterior border of the gland. Volumes calculated were related to the estimates of parenchyma as determined by dissection. The correlation coefficients between measures of parenchymal tissue by dissection and CT were: r^2= .80 for fat-free parenchyma, .78 for parenchymal protein and .62 for total parenchyma. Sorensen et al. (1987) concluded that due to the ability of CT to exclude extra-parenchymal tissue, mammary gland composition estimates had less variation than estimates determined by dissection and chemical analysis.

Similar work done as part of a nutritional study by Glaser et al. (1991) compared the mammary parenchyma of thirty-five, 770 kg Angus X Holstein heifers as determined by CT or dissection and chemical analysis. At slaughter, mammary glands were collected and parenchymal tissue of the left mammary gland was dissected from extra-

parenchymal tissue and both tissues weighed. The right mammary gland was scanned by CT to quantify parenchymal tissue volume using the technique

described by Sorensen et al. (1987). Glaser et al. (1991) concluded that CT was an effective method to quantify parenchymal tissue volume of the mammary gland based on comparison to parenchymal tissue weight determined by dissection, however, correlation coefficients were not reported. Glaser et al. (1991) reported left-gland values of 1,225.6 g total weight , 376.8 g parenchymal weight and a total parenchymal volume of 1,177.9 cm^3. Sorensen et al. (1987) reported respective whole udder measurements of 1277 g, 455 g and 1231 cm^3 . These variations in values between studies may be due to differences in breed, age and size of animal, however, additional studies using CT to quantify mammary composition are warranted.

While mammary gland dissection and chemical analysis provide reliable estimates of mammary gland composition, technology in the form of CT could allow for more accurate composition estimates to be determined. Computed tomography work to date has been limited by the size of equipment and thus has been conducted with dissected udders of cattle. The question of correlating in vivo mammary gland composition and milk production has yet to be addressed. Given their smaller size, it may be possible to scan the mammary glands of live ewes and correlate composition and developmental data with future milk production.

Measuring milk production in the ewe

Accurately measuring milk production in the ewe is a difficult task. Barnicoat et al. (1949) investigated several methods of milking ewes including hand and machine milking, pituitrin injections and estimating ewes' milk yields from the milk intakes of their lambs. These researchers found hand-milking to be tedious and incompatible with the complete evacuation of milk from the mammary gland and determined that machine milking was impractical, although the reasons why were not stated. Barnicoat et al., (1949) reported that the intravenous injection of the pituitary hormone commercially known as 'Infundin', while effective at stimulating milk let down in 14 of 17 ewes, was unreliable. Finally, Barnicoat et al. (1949), determined that the most practical, effective and reliable means of measuring milk production in the ewe was based on estimating ewes' milk yields from the milk intake of their lambs, a method commonly known as weigh-suckle-weigh.

More recently, Henry and Benson (1989) compared machine milking and a modified weigh-suckle-weigh method of measuring milk production in the ewe. Milk production was measured using weigh-suckle-weigh and machine milking every three days from 6 ± 1 to 63 ± 1 days of lactation (Henry and Benson, 1989). The modified weigh-suckle-weigh method differed from that described by Barnicoat et al. (1949) in that ewes were separated from lambs for 3 hours (h) by

placing them in adjoining pens. After 3 h, lambs were returned to ewes and allowed to nurse. Lambs were then separated for an additional 3 h, after which they were weighed, allowed to nurse and re-weighed. In this manner, 3 h milk production was determined. The machine milking procedure used commercial sheep milking equipment to evacuate the udder. Ewes were given 10 I.U. intravenous oxytocin and were then machine milked. Initial milk was discarded and ewes were separated from lambs for 3 h. After 3 h, ewes were again injected intravenously with oxytocin, machine milked to evacuate the udder and milk was weighed to determine 3 h milk production. Benson and Henry (1989) determined that the average milk production of 13 cross-bred ewe lambs was similar regardless of method, averaging 2.6 and 2.8 kg/d for weigh-suckle-weigh and machine milking, respectively. In addition, machine milking produced a more consistent lactation curve than did weigh-suckle-weigh. Henry and Benson (1989) reported that during the first 30 days of lactation, the lambs did not always evacuate the udder during the weigh-suckle-weigh procedure, which would explain the inconsistencies in the lactation curve.

While Barnicoat et al. (1949) determined that weigh-suckle-weigh was the most effective means of measuring milk production in the ewe, there are definite drawbacks to using this procedure. Should a lamb defecate or urinate after suckling but prior to being weighed, the milk production measurement becomes

less accurate. In addition, as reported by Henry and Benson (1989), a lamb may not completely evacuate the udder during a suckling bout, thus, the measurement becomes that of milk consumed by the lamb and not milk produced by the ewe. Since the researcher has little or no control over either of these situations, and based on the work by Henry and Benson (1989), machine milking is considered a more reliable method of measuring milk production of the ewe.

Plane of Nutrition and Future Milk Production in Replacement Females
Dairy Cattle

Effect of pre-pubertal nutrition on mammary development and composition in the dairy heifer

It is well documented that a high plane of nutrition prior to puberty impairs development of parenchymal tissue in dairy heifers (table 3). Sejrsen et al. (1982) used12 pre- and12 post-pubertal Holstein-Friesian heifers to investigate the effect of plane of nutrition on mammary development. Heifers were randomly assigned to either an ad libitum or restricted dietary treatment and were fed a 60:40 concentrate: forage ration for average daily gains of 1, 218 g and 613 g, respectively. Feed intake of restricted heifers was 55% that of ad libitum intake heifers and all animals were slaughtered at 320 kg body weight. Pre-pubertal heifers fed ad libitum had 23% lower mammary secretory tissue weights and 32% less DNA compared to heifers on restricted feeding. Post-pubertal heifers showed no treatment difference in mammary secretory tissue development,

Table 3: Effect of a high plane of nutrition on mammary composition in pre-pubertal dairy cattle.

Study	Initial Age (mos.)	Body Wt. at Slaughter (kg)	Diets	Daily Rate of Gain (g)	Results
Sejrsen et al., 1982	7	320	60:40 conc: forage	Restricted; 613 Ad Lib; 1218	-23% lower mammary secretory tissue weight -32% lower DNA content in ad lib fed heifers
Harrison et al., 1983	3	377	dried lucerne and barley	low (L); 570 medium (M); 760 high (H); 1180	-parenchymal weight and weight as a percent of total gland weight lower in H than L.
Petitclerc et al., 1983	na	340	alfalfa-brome haylage, corn silage, high moisture corn	L; 700 H; >1000	-mammary parenchymal DNA concentration and total DNA decreased in H
Stelwagen and Grieve, 1990	6-8	L; 378 M; 420 H; 447	cracked corn, chopped alfalfa grass hay	L; 611 M; 737 H; 903	-Increasing plane of nutrition resulted in fatter mammary glands with decreased concentration of DNA.
Capuco et al., 1995	7.3	LA; 335 HA; 338 LC; 329 HC; 331	alfalfa silage (A) or corn silage (C)	LA; 766 HA; 974 LC; 792 HC; 1011	-Total mammary parenchymal DNA and RNA was reduced in HC heifers. -Mammary parenchyma in HC heifers contained more adipocytes and a lower volume of epithelial cells.

suggesting that pre-pubertal dietary treatment was critical to mammary growth (Sejrsen et al., 1982).Harrison et al. (1983) used 19 British-Friesian or British-Friesian-Ayrshire cross heifers to analyze size and composition of mammary glands of dairy heifers reared at different rates of live weight gain. Eleven heifers were fed ad libitum intakes of a barley-based diet from 3 to 15 months of age. These heifers were bred to calve at 19 months of age and were considered the rapid-rearing group, gaining 1.1 $kg \cdot d^{-1}$ from 3 to 9 months of age and .7 $kg \cdot d^{-1}$ during gestation. Eight calves were reared on summer pasture and hay plus concentrate in the winter, and were bred to calve at 28 months of age. Pasture reared calves gained .55 kg/day before conception , .65 kg/d during gestation , and were considered the conventionally-reared group. Following first calving, all heifers were managed similarly for five lactations for the rapid rearing group and four for the conventional group. Cows were then slaughtered at 276 ± 2.6 days of their final pregnancy and mammary glands removed and halved for analyses. Harrison et al. (1983) reported half-mammary gland weights of 15.9 kg and 22.1 kg for rapid and conventionally reared heifers, respectively. Conventionally-reared animals had a greater amount of secretory tissue both by weight (14.8 kg vs. 8.79 kg) and when expressed as a percent of the udder (67.3% vs. 54.6%). Harrison et al. (1983) do not report body weights of either group at any point during the study. It is possible that the difference in mammary weight is related

to a difference in total body weight at conception, since conventionally-reared heifers were bred 10 months later than the rapidly-reared group and thus may have been larger at breeding. Based on daily gains reported for each group at each phase, the conventionally-reared animals were at least 30 kg heavier than the rapidly-reared animals. Regardless of total body weight, however, the fact that rapid-rearing heifers had less secretory tissue both by weight and as a percent of the udder lends support to the theory that rapid-rearing prior to puberty is detrimental to mammary secretory cell development.

In a related experiment, Harrison et al. (1983) examined mammary gland weight and composition in heifers reared at low (L), .57 kg/d, medium (M), .76 kg/d or high (H), 1.18 kg/d rates of gain from 3 to 11 months of age. While total gland weight was related to live-weight gain, dissected mammary parenchyma was heavier in heifers on treatment L, .29 kg than those on treatment H, .17 kg.

Petitclerc et al. (1983) examined the effect of photoperiod and plane of nutrition on carcass composition and mammary development in Holstein heifers . In a 2 X 2 factorial design, 20 pre-pubertal heifers were assigned to four treatment groups. The main effects included: photoperiods of 8 hours light: 16 hours dark; 16 hours light: 8 hours dark; and low or high plane of nutrition. Heifers on the low plane of nutrition were fed to gain .7 kg/day, while those on the high plane were fed at ad libitum intakes to achieve gains of more than 1 kg/ day. Heifers were on treatments for approximately 200 days, when they were

slaughtered and left mammary glands removed for analysis. Results showed that only level of nutrition and not photoperiod influenced development and composition of mammary parenchymal tissue. Heifers on the high plane of nutrition had reduced mammary concentration of DNA (1.77 mg/g vs. 1.92 mg/g), and reduced total amounts of DNA in mammary parenchymal tissue (172 vs. 227 mg/100 kg body weight) when compared to those on the lower plane of nutrition.

Stelwagen and Grieve (1990) used 41 6 to 8 month-old Holstein and six Holstein crossbred heifers to determine the effect of plane of nutrition on growth and mammogenesis prior to and during puberty. Heifers were fed a diet of cracked corn and chopped alfalfa grass hay to gain .61 $kg \cdot d^{-1}$ (L), .73 $kg \cdot d^{-1}$ (M) or .90 $kg \cdot d^{-1}$ (H). Heifers were divided into a Production (P, n=24) group that went through lactation and a Slaughter group (S, n=23)that was slaughtered after 279 days on trial. Mammary glands were removed from S heifers and frozen for analysis of fat, crude protein, DFFT and total DNA. Increased plane of nutrition resulted in fatter mammary glands with decreased concentrations of DNA. Heifers on the H and M diets had 129% and 57% more mammary fat than L heifers; however, total DNA did not differ among treatments. Unlike the Petitclerc et al. (1983), Sejrsen et al. (1982) and Harrison et al. (1983) studies, Stelwagen and Grieve (1990) measured total mammary composition and did not separate parenchymal and extra-parenchymal tissue, which may explain the lack

of difference in total mammary DNA between treatments. Sejrsen et al. (1982) also saw no difference in total mammary DNA between pre-pubertal heifers fed restricted or ad libitum intakes; however, there was 47% more parenchymal DNA in the restricted than ad libitum fed group. Both the Sejrsen et al. (1982) and Stelwagen and Grieve (1990) studies suggest that pre-pubertal heifers on restricted intakes exhibit greater secretory cell proliferation, while ad libitum fed, pre-pubertal heifers exhibit greater cell proliferation in the stromal portion of the mammary gland, to a large extent in the form of fat.

Capuco et al. (1995) provide further evidence that a high plane of pre-pubertal nutrition influences mammary development in the dairy heifer, however, diet composition was also shown to have an effect. In a 2 X 2 factorial design, 116 Holstein heifers in a 2 year study were randomly assigned to diets of alfalfa silage or corn silage at high or low rates of gain. High-group heifers were fed their respective ration to gain .96 $kg \cdot d^{-1}$, while low-group animals were fed to gain .73 $kg \cdot d^{-1}$. Each year, four heifers were slaughtered prior to onset of treatment to provide mammary composition data for comparison. Of the remaining animals, heifers were removed from dietary treatment upon reaching 325 kg and having had two or more estrous cycles. At this time, four heifers per year were slaughtered and mammary glands removed for analysis. The remaining animals were managed similarly, all were bred and subsequent milk production recorded. Total mammary parenchymal DNA and RNA were reduced

in heifers reared at a high rate of gain on corn silage but not alfalfa silage. As in the Sejrsen et al. (1983) and Johnsson et al. (1985) studies, growth hormone levels were reduced in animals reared at a high rate of gain, but Capuco et al. (1995) saw this reduction only in those animals on the high corn silage diet. While these data agree with previous work showing that a high plane of nutrition prior to puberty limits mammogenesis, Capuco et al. (1995) saw no difference in first lactation milk production across treatments. This may have been due to a compensatory growth effect in the mammary gland during gestation, thus making up for the lack of parenchymal tissue developed during the pre-pubertal allometric growth phase.

Finally, Niezen et al. (1996) used twenty 118 kg Holstein heifers to measure the effect of plane of nutrition before and after 200 kg of body weight on mammary development in Holstein heifers. Heifers were randomly assigned to four dietary treatments in a 2 X 2 factorial design. Heifers were fed a corn based total mixed ration for High (1 kg/day) or Low (.7 kg/day) rates of gain during two time periods: Period One, from 118 kg to 200 kg of body weight and Period Two, from 200 kg to slaughter in the middle of the luteal phase following the third estrus. At slaughter, mammary glands were removed and frozen for analysis of DM, DFFT, CP, DNA and RNA. Heifers on the low plane of nutrition throughout the trial had a greater concentration of mammary DNA and RNA; however, total mass of DNA and RNA did not differ among treatments, as in

studies by Sejrsen et al. (1982) and Stelwagen and Grieve (1990). Heifers on the high plane of nutrition during Period Two had heavier mammary glands with increased amounts of protein, fat and DFFT. The entire glands were analyzed in this study, as opposed to the dissection of parenchymal tissue and stroma for separate analysis. Consequently, while the mammary glands of heifers on the high plane of nutrition in Period Two were heavier, the additional protein, fat and DFFT weights may have been part of the stroma and not parenchymal tissue. Conclusive results regarding parenchymal composition may not be drawn from this study; however, once again a relationship is shown between pre-pubertal dietary treatment and mammary development.

There is much evidence to support the theory that pre-pubertal plane of nutrition affects mammary composition in the dairy heifer. Heifers on a high plane of pre-pubertal nutrition may have heavier mammary glands (Niezen et al., 1996) and similar amounts of total mammary DNA (Stelwagen and Grieve, 1990), however, heifers fed for limited growth have greater amounts of parenchymal weight (Sejrsen et al.; 1982, Harrison et al., 1983) and parenchymal DNA (Petitclerc et al., 1983). Mammary glands of heifers reared on a high plane of pre-pubertal nutrition, while larger, tend to be fatter than those of heifers reared on limited intakes during the same period (Stelwagen and Grieve, 1990), which may account for the lack of difference in total mammary DNA in some studies.

Effect of pre-pubertal nutrition on future milk production in the dairy heifer

A high plane of nutrition prior to puberty has a negative effect on the development of mammary secretory tissue in the dairy heifer. Milk production is limited by the number of secretory cells present in the mammary gland (Tucker, 1969), however, the relationship between plane of nutrition during development and future milk production is not clear.

Gardner et al. (1977) studied the effect of accelerated growth and early breeding on reproductive and productive characteristics, including milk production, of Holstein heifers. Twenty-four Holstein heifers (Accelerated Group, A), were fed a concentrate-forage ration at ad libitum intakes from 91 kg body weight until verification of pregnancy. Group A heifers were bred at second estrus upon reaching 305 kg. A second group of 24 heifers (Standard Group, S) were fed a roughage ration and were bred at 15 to 16 months of age. Group A heifers gained 1.1 $kg \cdot d^{-1}$ as compared to .8 $kg \cdot d^{-1}$ gained by group S. Heifers were bred at 319 kg and 9.6 months of age and 392 kg and 16.8 months of age for groups A and S, respectively. Weight at first calving did not differ by treatment. Heifers fed a roughage ration to gain .8 $kg \cdot d^{-1}$ to 15 months of age produced 5, 415 kg of milk in the first 100 days of lactation as compared to 4,436 kg produced by A heifers during the same period. There were no differences in milk production in the second, third or fourth lactations.

Little and Kay (1979) also examined the effect of rapid-rearing on the subsequent milk yields of dairy heifers. One hundred and ten British-Friesian and British-Friesian cross heifers were randomly assigned to three treatment groups: Group A, gains of greater than 1 $kg \cdot d^{-1}$ from 13 to 19 weeks of age, bred at 302 kg of body weight; Group B, fed as Group A, but exposed for breeding at 443 kg of body weight; and Group C, reared on summer grazing and winter concentrates to gain .74 $kg \cdot d^{-1}$ and bred at 353 kg body weight. Little and Kay (1979) measured milk yields of all heifers for the first four lactations and concluded that milk yields were lower in all lactations of rapidly-reared animals, regardless of age and body weight at conception.

In a study designed to induce compensatory growth during the pre-pubertal, pubertal and late gestation stages of mammary development, Park et al. (1987) assigned 20 5.5 month-old Holstein heifers to a Control or Treatment group. The Control group was fed to meet NRC recommendations for growing heifers (.45 $kg \cdot d^{-1}$ gain). The Treatment group was fed on a schedule beginning with a 3 month dietary restriction of 15% below NRC requirements for dairy heifers and alternating with a dietary realimentation of 40% above NRC for 2 months. Dietary treatments were alternated in this fashion for an additional 5 month restriction, 2 month realimentation, 5 month restriction, 2 month realimentation. In a subsequent paper, Park et al. (1989) reported that Treatment heifers produced 8,715 kg of milk expressed as the mean of 4

lactation records averaging 7.6 cows per record. Control heifers produced 7,913 kg of milk expressed in the same manner. Based on the format in which Park et al. (1989) reported these data, it is impossible to determine if milk yields in Treatment cows remained high through all 4 lactations, or if yields were higher in the first lactation but not different in subsequent lactations as reported by Gardner et al. (1977). Park et al. (1989) reported that milk yields were approximately 10% greater in Treatment cows than Control cows, although the exact nature of the difference from lactation to lactation is unknown.

Capuco et al. (1995) evaluated the influence of pre-pubertal dietary regimen on growth, mammary composition and milk production in Holstein heifers. In a 2 X 2 factorial design, 116 heifers were randomly assigned to either an alfalfa or corn silage diet and were fed at High (.95 $kg \cdot d^{-1}$) or Low (.73 $kg \cdot d^{-1}$) rates of gain until the third estrus, when they were bred. Following breeding, all heifers were managed similarly. As reported previously in this review, high rates of gain prior to puberty had a negative effect on mammary development in a subset of heifers slaughtered during this study. This impairment did not alter future milk yields, however, as Capuco et al. (1995) reported no difference in milk yield across treatment groups.

While a high plane of pre-pubertal nutrition has a negative effect on parenchymal weight and parenchymal DNA (Sejrsen et al., 1982), this difference in secretory tissue does not consistently result in a difference in future milk

production (Gardner et al., 1977, Park et al., 1989, Capuco et al., 1995).

Beef Cattle

To optimize lifetime productivity, beef heifers must conceive by 15 months of age and calve by 24 months of age (Lesmeister et al., 1973). Heifers that calve early in their first breeding season tend to calve early throughout their lifetimes, thus improving calf weight at weaning and subsequently, profit. Much of the nutritional research done with beef heifers has focused on achieving rapid pre-pubertal weight gains to limit days to puberty, often through the use of pre-weaning creep feeding practices. By decreasing the number of days to puberty, heifers have an increased chance for conception at 15 months of age, however, mammary development may be compromised.

Effect of pre-pubertal nutrition on mammary development and composition in the beef heifer

Little work has been done to examine the relationship between limiting days to puberty in beef heifers and it's effect on mammary development and future milk yield. Glaser et al. (1991) studied the effects of continuous versus intermittent growth on mammary gland composition. Thirty-five, 6-month-old Angus X Holstein heifers were assigned to four treatments as part of a study to examine the effects of Bovine Somatotropin (bsT) and intermittent growth on the bovine mammary gland. Treatment groups consisted of: 1) continuous growth and carrier; 2) continuous growth and bST; 3) intermittent growth and carrier;

and 4) intermittent growth and bST (Glaser et al., 1991). Continuous growth heifers were fed a 58% concentrate diet to gain .80 $kg \cdot d^{-1}$ throughout the experiment. Intermittent growth heifers underwent two successive growth cycles consisting of a 3-month restricted growth period during which heifers were limit fed to gain .23 kg/d and a compensatory growth phase during which heifers were fed a 90% concentrate diet at ad libitum intakes. When all animals reached an average weight of 386 kg, they were slaughtered and udders were removed and frozen for analysis (Glaser et al., 1991). Heifers fed for intermittent growth without bST had smaller udders by weight than heifers fed for continuous growth without bST, 1225 g vs. 1508 g, respectively. The udders from intermittent growth heifers had greater amounts of parenchyma by weight, 377 vs. 348 g for intermittent growth and continuous growth heifers respectively. Mammary glands from continuous growth heifers contained a greater percentage of lipid than did mammary glands from the intermittent growth heifers, 44.4% versus 41.45, respectively (Glaser et al, 1991).

Buskirk et al. (1996 a) studied the effect of providing ad libitum creep feed prior to weaning in beef heifers. Twenty-eight crossbred heifer calves nursed their dams while on pasture and received either ad libitum access to creep feed (85% cracked corn, 15% soybean meal) or no creep feed for 112 days. After the 112 day treatment period, heifers were managed alike through the weaning of their first calf at an average of 162 d post-partum. All heifers were slaughtered

within 18 hours of calf weaning and mammary glands removed for analysis. Heifers receiving creep feed had lower mammary wet weights (7,370 g vs. 8,860 g), lower amounts of DFFT (841 g vs. 1,041 g) and DNA (7,279 vs. 9,594 mg) than those without creep, respectively. The results of both the Buskirk et al. (1996) and Glaser et al. (1991) studies support the theory that by feeding beef heifers for rapid growth, the number of secretory cells in the mammary gland may be reduced, potentially reducing future milk production and subsequent calf gain.

Effect of pre-pubertal nutrition on future milk production in the beef heifer

While the number of studies examining the relationship between pre-pubertal nutrition and mammary composition in beef heifers is limited, more work has been done to study the effect of pre-pubertal nutrition on future milk production in beef heifers.

A 21 year study was conducted to evaluate the effects of creep feeding on subsequent cow productivity (Martin et al., 1981). Martin et al. (1981) concluded that 210 cows creep-fed between 4 and 7 months of age produced fewer total calves weaned, lighter calf birth-weights and lighter 120 and 210 day calf weights than cows that were not creep-fed. Martin et al. (1981) do not report the nutrient density of the creep feed used, however, when weaned at 210 days of age , creep-fed calves were 15 kg heavier than non-creep-fed calves and creep-fed female calves were 10 kg heavier than non-creep-fed female calves. Consequently, heifer calves receiving creep-feed were on a higher plane of

nutrition during the pre-pubertal allometric mammary growth phase , which may have impaired development of mammary parenchymal tissue and ultimately, future milk production of these cows. This theory is supported by the high correlation between milk production of the dam and weaning weight of the calf (Martin et al., 1982), as creep-fed cows produced calves with lower weaning weights.

Similarly, Hixon et al., (1982) reported that first-calf heifers creep-fed while nursing their dams had lower 24-hour milk yields at 120 days of lactation than those that had not had access to creep feed. Sixteen Angus and sixteen Hereford heifer calves were used to examine the effects of creep feeding and monensin on reproductive performance and lactation in beef heifers. Ninety days prior to weaning, one half of each group and their dams were assigned to an alfalfa-smooth bromegrass pasture and creep feed was made available to the calves. Although intakes were not measured, creep-fed heifer calves had heavier (219 kg) weaning weights than non-creep-fed calves (202 kg) when adjusted for age of dam and to an equal age at weaning (Hixon et al., 1982). After weaning, all heifers were managed alike throughout the experiment. Following parturition by the original heifer calves, two successive 12-hour milk yield measurements were taken on days 60 and 120 of lactation. Calves were separated from their dams for 12 hours and returned and allowed to nurse before being separated again. At 12 and 24 hours following nursing, dams were

given an i.m. injection of oxytocin and were machine milked to evacuate the udder. Milk yield data from the two 12 hour intervals was used to determine 24 hour milk yield. One hundred and twenty day milk-yield was greater in non-creep-fed females (4.5 kg) as opposed to creep-fed females (3.5 kg). In addition, 60-day milk yield data were numerically greater in non-creep-fed calves (5.0 kg) as opposed to creep-fed calves (4.1 kg). The milk yield values by treatment group provide support for the theory that a high plane of nutrition prior to puberty may impair future milk production and ultimately, calf gain and profit.

Buskirk et al., (1996b) evaluated the relationship between length of time heifers receive creep feed and subsequent heifer fertility and milk production. Ninety heifer calves were randomly assigned to receive creep feed for 0, 28, 56 or 84 days prior to weaning and average daily gains of .58, .42, .69 and .91 $kg \cdot d^{-1}$, respectively, were reported. Subsequent 52 day milk production of creep-fed heifers decreased linearly as time receiving creep feed increased, but there was no treatment effect on milk production at 102 and 151 days of lactation.

Based on the results of these studies, creep-feeding of replacement beef heifers has a detrimental effect on subsequent milk production and calf performance. This is likely due to a decrease in growth of parenchymal tissue during the allometric mammary growth phase prior to puberty.

In a study that conflicts with the theory that a high plane of pre-pubertal nutrition has a negative effect on subsequent milk yield, Marston et al. (1995) found that milk production was not altered by the limit-feeding of a high concentrate diet for 60 days prior to breeding. Marston et al. (1995) used 100 7-month-old, spring born heifers to evaluate the effect of post-weaning diet on age and weight at puberty, and milk production in replacement heifers. Three groups of heifers grazed dormant native pastures and were supplemented with either .9 $kg \cdot d^{-1}$ 40% CP soybean meal or a high (2.8 $kg \cdot d^{-1}$) or low (1.7 $kg \cdot d^{-1}$) level of a 20% CP supplement (HIGH-20 or LOW-20). A final group was fed .9 kg/d of soybean meal from October to February and then received a high concentrate diet in drylot to weights equaling those of the HIGH-20 heifers on May 1, when breeding began. The heifers calved from February to April. In the last week of April, milk production was calculated for one 24-hour period using the weigh-suckle-weigh method at 8 hour intervals. These researchers found that limit feeding a high concentrate diet for 60 days prior to breeding does not effect subsequent milking ability. Reported milk production in this study is based on the weigh-suckle-weigh method at one point in heifers at various stages of lactation. Weigh-suckle-weigh can be an unreliable means of determining milk production, since the calf may drink, urinate or defecate after suckling but prior to being weighed, thus altering calf weight. This variation could account for the lack of difference in milk production across treatments.

Ewes and Does

Effect of pre-pubertal nutrition on mammary development and composition in ewes and does.

While the evidence is not as abundant as in dairy or beef cattle, level of pre-pubertal nutrition appears to affect mammary development in the ewe. McCann et al. (1989) fed eight Suffolk ewes a 60:40 concentrate : forage finishing ration to gain 239 $g \cdot d^{-1}$ from weaning at 42 days of age to puberty at 199 days of age. Mammary area of these ewes was compared to that of eight Suffolk ewes fed a 40:60 concentrate: forage ration to gain 179 $g \cdot d^{-1}$ from weaning at 42 days of age to puberty at 206 days of age. Lambs were euthanized at 11± 1 day after exhibiting first estrus and half the mammary gland was immediately infused with a red vinyl solution via the teat canal. The teat was then tied off and the udder skinned and removed at the median suspensory ligament. Udder halves were fixed, sliced and photographed . Gross duct area, and gland and cistern areas were measured with a planimeter and fat pad area was estimated by subtracting gross duct area from total gland area. McCann et al. (1989) determined that mammary gland fat pad area was greater for ewes on the finishing diet than on the growth diet; 183.2 cm^2 and 159.4 cm^2 respectively , however, there was no difference in duct area. This study does not provide any information as to the composition of the mammary gland, however the results with respect to total gland size are similar to those found by Sejrsen et al. (1982)

and Capuco et al. (1995) in that a higher plane of nutrition resulted in a larger mammary gland.

Johnsson and Hart (1985) also determined that trimmed mammary fat pad weight, 29.99 g was greater in ewe lambs fed a high energy, high protein diet to gain 220 $g \cdot d^{-1}$ than in those fed restricted intakes of the same diet to gain 110 $g \cdot d^{-1}$, 14.74 g from 4 to 20 weeks of age. As in the McCann et al. (1989) study, ewes on a higher plane of nutrition prior to puberty had larger mammary glands, however, Johnsson and Hart (1985) determined that these ewes had lower amounts of fat pad containing parenchyma, 8.21 g when compared to that of ewes on the lower plane of nutrition, 9.57g. Ewes fed to gain 110 $g \cdot d^{-1}$ prior to puberty had greater amounts of parenchymal DFFT, 844 mg, total DNA and RNA, 32.3 mg and 28.5 mg, than ewes fed to gain 220 $g \cdot d^{-1}$; 623 mg, 25.9 mg and 23.5 mg, respectively. In contrast, ewes fed to gain 220 $g \cdot d^{-1}$ prior to puberty had 623 mg of parenchymal DFFT, total DNA and RNA; 25.9 and 23.5 mg respectively.

Finally, work by McFadden et al. (1990) determined that restricting feed intake to provide a gain of 112 $g \cdot d^{-1}$ resulted in a higher percentage of parenchymal tissue and a lower percentage of fat pad when compared to ewes fed ad libitum amounts of the same diet. McFadden et al. (1990) concluded that because the mammary glands of the restricted ewes were smaller by weight, there was no difference in parenchymal weight between treatments. In addition,

parenchymal dry, fat-free tissue was actually reduced in restricted ewes as compared to ad libitum fed ewes (McFadden et al., 1990).

High pre-pubertal intakes also appear to have a negative effect on the development of mammary parenchyma in the goat. Ten 6-week- old French Alpine kids were paired on the basis of body weight and were fed either ad libitum or restricted intakes of Jersey cow's milk (Bowden et al., 1995). The restricted kids received 70% of their pair-mate's ad libitum milk intake for 4 weeks and then 50% of their pair-mate's milk intake for 9 weeks. The kids fed at ad libitum intakes had larger mammary glands by weight and greater amounts of adipose tissue than those fed at restricted intakes. The restricted kids had more DNA and protein per gram of mammary gland indicating greater cell number.

These studies suggest that as in dairy (Capuco et al., 1995) and beef (Glaser et al., 1991) cattle, a high plane of pre-pubertal nutrition affects mammary composition in ewes and does. Ewes and does fed ad libitum dietary intakes prior to puberty have larger mammary glands by weight (McCann et al., 1989, Bowden, et al., 1995) but more total mammary adipose tissue (McFadden , 1990) and less parenchymal tissue (Johnsson and Hart, 1985) than ewes and does fed restricted intakes prior to puberty.

Effect of pre-pubertal nutrition on future milk production of the replacement ewe

Milk production is limited by the number of cells synthesizing milk (Tucker, 1969). A high pre-pubertal plane of nutrition will decrease the number

of parenchymal cells, which could potentially reduce future milk production. Some studies have shown reduced milk production in cattle as a result of pre-pubertal plane of nutrition (Gardner et al., 1977, Park et al., 1989) however, little work has been done to examine the effect of pre-pubertal plane of nutrition on subsequent milk production in the ewe.

Umberger et al. (1985) determined that early weaned ewe lambs fed for accelerated gain produced less milk than those fed for moderate gain. In three trials, 113 Suffolk and Suffolk crossbred ewes were weaned at 20 kg body weight and were randomly assigned to one of two pre-breeding treatments. Thin (T) group ewes were fed an alfalfa hay/ground corn diet to gain 30 days, when they were placed on pasture. Fat (F) group ewes were fed the alfalfa/ground corn diet 30 days when they were also placed on pasture; however, F ewes were allowed ad libitum intakes of ground corn while on pasture. Gains from weaning to breeding for T and F ewes were: 131, 217; 112, 173; 113, 204 $g \cdot d^{-1}$ in trials 1,2 and 3, respectively. Four hour milk production was determined at 20, 40 and 60 days of lactation and was converted to 24 hour milk production. At 20 and 40 days of lactation on the first trial, milk production was determined using the weigh-suckle-weigh procedure. At 60 days of lactation and for all subsequent milk yield estimates, 4 hour milk production was estimated using a machine milking technique. Twenty-four hour milk production at 20, 40 and 60 days of lactation was 1,482, 1,571, 1614 $g \cdot d^{-1}$ for T ewes; and 1183, 1373 and 1321 $g \cdot d^{-1}$

for F ewes, in trials 1,2 and 3, respectively. A significant difference in milk production was only found in trial 3.

McCann et al. (1985) found that ewe lambs fed a finishing diet to gain 239 $g \cdot d^{-1}$ from weaning to puberty produced less milk than ewes fed a growing diet to gain 179 $g \cdot d^{-1}$ during the same period. Four-hour milk production was measured at 25 days of lactation using a machine milking technique. Ewes on the pre-pubertal finishing ration produced 283 g of milk per 4 hours as compared to 310 g of milk produced by ewes on the pre-pubertal growing diet. Milk production was measured at one point in lactation during this study, and consequently, further research is needed to determine if ewes on a high plane of pre-pubertal nutrition produce less milk over time than those on restricted intakes prior to puberty.

The McCann et al. (1985) and Umberger et al., (1985) studies suggest that it is possible to affect future milk production in the ewe by manipulating pre-pubertal plane of nutrition. While these studies primarily measure milk production in the ewe using a machine milking technique, measurements are taken at one (McCann et al., 1985) or three (Umberger et al., 1985) points of time during lactation. More research is required to determine if milk production differs over the course of lactation between ewes reared on a high pre-pubertal plane of nutrition and those reared on restricted intakes during the same period.

Chapter Three

MATERIALS AND METHODS

The primary objective of this study was to determine the effect of a pre-pubertal dietary restriction and subsequent realimentation on future milk production of the replacement ewe. Specific objectives were to 1) determine the effect of pre-pubertal dietary restriction and subsequent realimentation on future milk production of ewes; and 2) to compare growth hormone profiles between dietary restricted and ad libitum fed ewes.

Materials and Methods

This study was conducted under the approval of the Michigan State University All University Committee on Animal Use and Care (AUF: 07/95-100-00).

Animals. Fifty-three Dorset X Suffolk X Rambouillet ewes born and reared as twins, weighing an average of 25 kg were weaned at 60 days of age and randomly assigned to Control (C, n=26) or Restricted (R, n=27) dietary treatment groups. Ewes were shorn, ear tattooed and paint branded for identification and were de-wormed with oral Tramisol (Pittman-Moore, Mundelein, IL). One ewe was removed from the R group for reasons unrelated to the study and one C ewe died during the growth phase as a result of a rectal prolapse. Ewes were housed by treatment in four pens, in which all ewes had

access to the feeders and were provided water and trace mineral salt free-choice. Straw was used as bedding.

Treatments. Control ewes had ad libitum access to alfalfa pellets (15% CP, 2.06 Mcal·kg^{-1} ME) throughout a 158 d growth phase (tables 4 and 5). Ewes were fed twice daily at approximately 0800 and 1600 h. Orts were measured once daily and amount of feed adjusted to produce 10% weigh-back. Restricted ewes were fed alfalfa pellets at quantities to support an average daily gain (ADG) of 113 g·d^{-1} for 120 d and were then allowed ad libitum access to alfalfa pellets for a 38 d realimentation period. All ewes were weighed weekly during the 158 d growth phase, and feed adjusted to provide desired ADG (figure 1). Following the 158 d growth period, all ewes were managed similarly and were fed approximately 2.0 kg alfalfa hay and .45 kg·d^{-1} of a concentrate mix (tables 4 and 5) during gestation. Lactating ewes nursing twins and singles were fed 1 kg·d^{-1} and .9 kg·d^{-1}, respectively, of a concentrate mix (tables 4 and 5) in addition to approximately 3.5 kg·d^{-1} of alfalfa hay.

Analysis of serum growth hormone concentration. At the conclusion of the 120 d restriction, eight ewes from each treatment were randomly chosen and fitted with sterile, indwelling jugular catheters. The following day, 5 mL blood samples were collected at 20-minute intervals for 6 h (0700 to 1300) following the removal of a 3 mL waste sample. Catheter patency was maintained between samples by flushing with 3.5% sodium citrate in sterile water. Samples were

Table 4: Composition of alfalfa pellet and concentrate mixes in experimental diets, DM basis

Growth Diet		Gestation Diet		Lactation Diet	
Ingredient (% of ration)		***Ingredient (% of ration)***		***Ingredient (% of ration)***	
dehydrated alfalfa meal	*100*	*oats*	*58*	*corn-cracked*	*78.2*
		corn	*26.5*	*soybean meal*	*12.3*
		soybean meal	*9.95*	*molasses*	*9.0*
		molasses	*3.32*	*salt-white*	*.5*
		vit. and min.	*2.5*		
Total	*100*		*100*		*100*

Table 5: Chemical composition of alfalfa pellet and concentrate mixes in experimental diets

	Growth	Gestation	Lactation
Dry matter (%)	96.9	96.6	95.5
Crude protein (%)	13.5	14.6	11.4
Neutral detergent fiber (NDF)	48	31.4	19.7
Acid detergent fiber (ADF)	31.6	14.5	5.73
Lignin (%)	8.3	2.74	1.74
ME (Mcal/kg)[1]	2.06	2.87	2.94

[1] Calculated from table values in Nutrient Requirements of Sheep (1985).

placed in borsilicate glass tubes and allowed to clot at room temperature. At the conclusion of the 6 h sampling period, blood samples were centrifuged for 20 minutes at 2,500 rpm and serum was poured into 12 X 75 mm plastic tubes, then fitted with caps and frozen for future analysis. Ewes were fed immediately following the 0800 h sample. Serum concentrations of growth hormone were determined according to Gaynor et al. (1995) using a radioimmunoassay technique.

Breeding. At the conclusion of the 158 d growth phase, estrus was synchronized in all ewes with a progestin implant placed in the ear and removed after 14 d. Vasectomized rams were introduced for 15 d for detection of estrus. Within 5 days of introducing vasectomized rams, the majority of ewes had been marked as being in estrus. The actual number of marked ewes was not recorded. All ewes were then randomly assigned within treatment to six pens; four containing nine ewes and two containing 8 ewes. Three Suffolk and three Dorset rams were randomly assigned one to each pen for one 20 d cycle of natural service breeding in an attempt to keep lambing dates and ultimately, stage of lactation as similar as possible for all ewes. Approximately 90 days post-breeding, ewes were trans-abdominally ultra-sounded (Pi-Medical Scanner) to determine pregnancy.

Parturition. Following parturition, each ewe and her lamb(s) were placed in 5' X 5' pen for 24 to 48 h. Ewes were given ad libitum access to hay and

water and ewes and lambs were paint branded for identification. Immediately following parturition, lambs were weighed and umbilical stumps were rinsed with iodine. Approximately 48 h post-partum, ewes nursing twin and ewes nursing single lambs were separated into two pens. Within 1 week of parturition, lambs were given a subcutaneous injection of Bo-Se, tails were docked, ear tags inserted and castration of males performed as necessary. Additional feeders were placed in each pen to allow all ewes access to feed.

Milk Production. Following parturition, ewes and lambs were weighed weekly and weights recorded. Three-hour milk production was measured twice weekly using a machine milking procedure (Henry and Benson, 1989). Ewes and lambs were separated and ewes were injected with 10 International Units (IU) intravenous oxytocin and machine milked to evacuate the udder. Milk was discarded and ewes and lambs remained separated for 3 h. Following the separation period, ewes were again injected with 10 IU intravenous oxytocin and machine milked to evacuate the udder. Milk collected from the second milking was weighed and recorded as 3-h milk production. Three-hour milk production was multiplied by 8 to estimate daily milk production.

Milk Composition. Milk samples were aliquotted once weekly for analysis of dry matter, protein and fat. Milk dry matter was determined in triplicate by oven drying a 3 mL sub-sample at 55° C for 48 h (AOAC, 1984). Milk crude protein was determined in triplicate by the Kjeldahl procedure using a Technicon

auto-analyzer system (AOAC, 1984). Milk fat was determined in triplicate using AOAC (1975) procedures adapted by Loudenslager (1984).

Statistical Analysis. Treatment, breed of ewe sire, and type of rearing effects were included in the model for the analysis of milk yield and composition, lactating ewe and their lamb weights. Milk yield and composition, growing ewe weights, lactating ewe and respective lamb weights were analyzed using Proc Mixed and SAS® (6.11). Data were analyzed with the following linear model ;

$$y_{ijk} = \mu + a_i + b_{ij} + t_k + e_{ijk}$$

where:

μ is the overall constant

a_i is the fixed effect of the i^{th} treatment, I=1,2

b_{ij} is the random effect of the j^{th} ewe effect associated in treatment j=1...56

t_k is the fixed effect of the k^{th} time, k=1...22

e_{ijk} is the random error associated with the b^{th} ewe in the a^{th} treatment at time k.

Ewes were randomly allocated to treatments and were assumed normally and independently distributed (NIID) with respect to treatment. The effects of time and the interactions of other factors with time were investigated with a first order auto-regressive covariance structure and homogenous variance for milk yield

and compositional data.

Overall mean concentrations of growth hormone were transformed by natural logarithm to eliminate heterogeneous variance and were analyzed using the ANOVA procedure of SAS®(6.11).

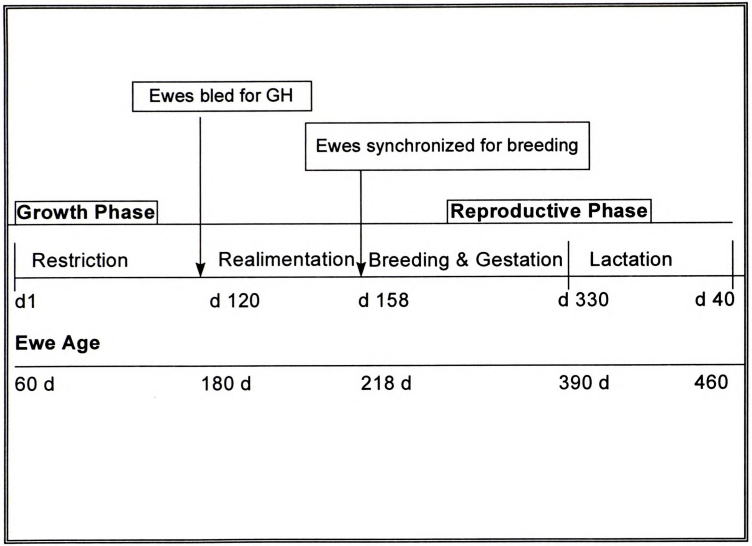

Figure 1: Timeline of project to determine the effect of a pre-pubertal dietary restriction on future milk production in ewes.

Chapter Four

RESULTS AND DISCUSSION

Growth Phase

Diet. The diet of C and R ewes consisted of alfalfa pellets throughout the restriction and realimentation periods. Metabolizable energy (ME) and crude protein levels consumed by C and R ewes during the restriction period are summarized in table 6. All ewes met or exceeded the NRC (1985) requirements for ME and crude protein, with the exception of R ewes who gained .11 $kg \cdot d^{-1}$ on less than their ME requirement during the 120 d restriction period. Restricted ewes grew from 26 ± .63 to 39.8 ± .92 kg and C ewes from 26 ± .63 to 52.7 ± .92 kg of body weight from the beginning of the trial to the end of the 120 day restriction period (figure 2). Thus, table 6 compares actual nutrients supplied by the diet to NRC (1985) requirements for replacement ewes from 30-60 kg, gaining from .12 to .23 $kg \cdot d^{-1}$

Restriction Period. Feed intake of R ewes was adjusted weekly to provide average daily gain (ADG) of .11 ± .06 $kg \cdot d^{-1}$. Restricted ewes consumed an average of 1.2 kg of alfalfa pellets daily during the 120 d dietary restriction period. Control ewes consumed an average of 2.6 kg of alfalfa pellets daily and gained .22 ±.06 $kg \cdot d^{-1}$ during the same period. At the conclusion of the restriction period, R ewes weighed 25% less than C ewes (P<.0001) (figure 2) .

Table 6: A comparison of average daily protein and energy consumed by Control and Restricted ewes during the 120 day restriction period

Treatment	Daily Feed Intake (kg)[1]	Daily ME Consumed (Mcal)[2]	Daily ME Required [3]	CP Consumed (g)[4]	CP Required (g)[5]
Control	2.6	5.36	2.8-3.3	405	132-185
Restricted	1.2	2.47	2.8-3.3	162	132-185

[1] Alfalfa pellets
[2] Alfalfa pellets, ME=2.06 Mcal/kg
[3] N R C (1985) requirements for replacement ewe lambs, 30 to 50 kg body weight, gaining .26 to .50 kg d^{-1}
[4] Alfalfa pellets, CP=13.5%
[5] N R C (1985) requirements for replacement ewe lambs, 30 to 50 kg body weight, gaining .26 to .50 kg d^{-1}

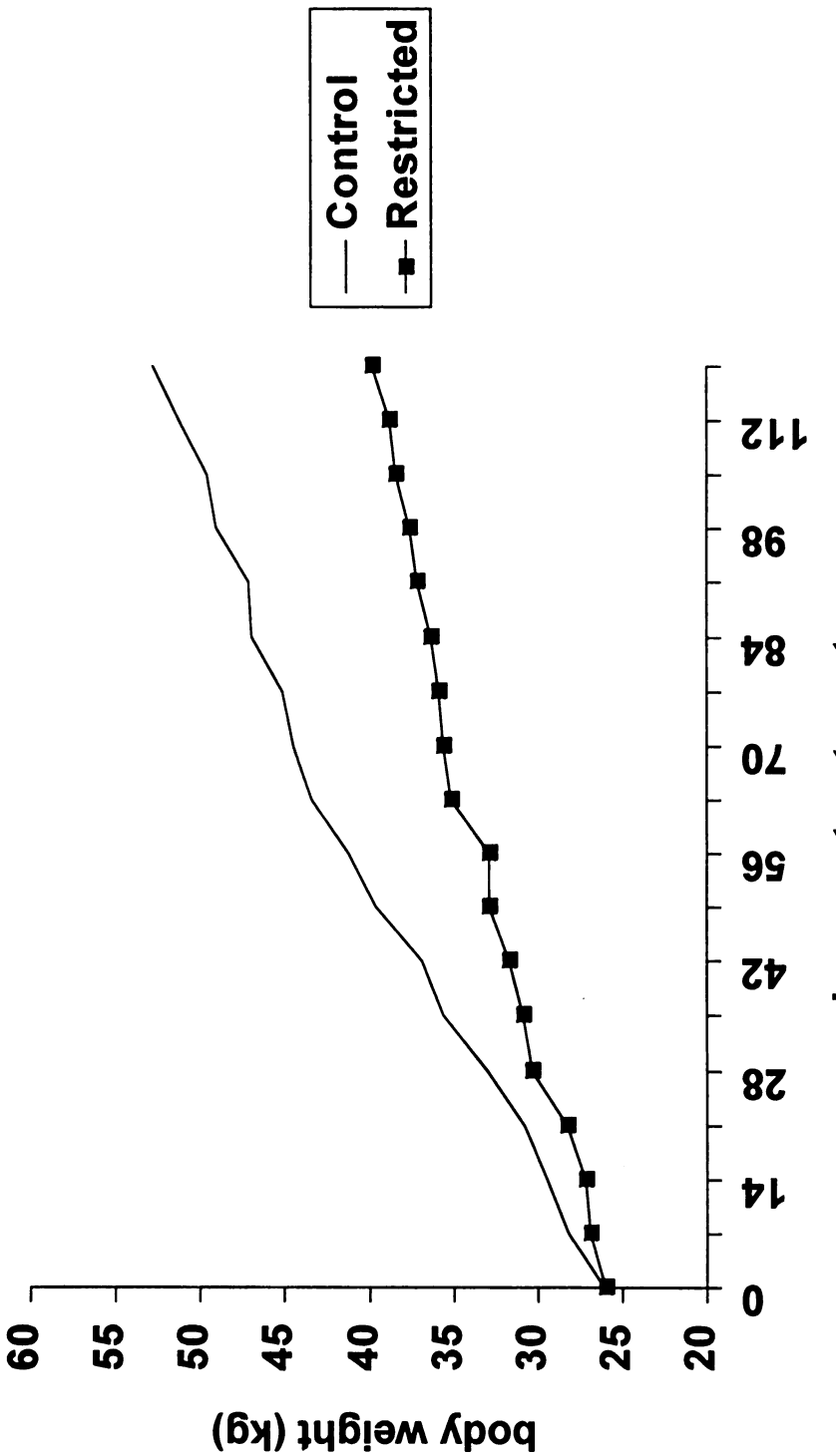

Figure 2: Ewe body weights during the 120 d restriction period

Realimentation Period. During the 38 d realimentation period, the feed intake of R ewes was increased approximately .10 $kg \cdot d^{-1}$ until ad libitum intakes were reached after 7 days. Restricted ewes consumed less feed (2.7 $kg \cdot d^{-1}$) than C ewes continuing on ad libitum intakes (3.0 $kg \cdot d^{-1}$) and exhibited greater ADG (.25 ±.03 $kg \cdot d^{-1}$) than C ewes (.17 ±.03 $kg \cdot d^{-1}$) during the realimentation period ($P<.0006$). While R ewes exhibited greater efficiency of gain than C ewes, their rate of gain was not sufficient to bring them to body weights equal those of C ewes at the conclusion of the realimentation period.

Growth Phase. The growth phase is defined as the 120-d restriction period plus the following 38-d realimentation period. Restricted ewes gained an average of .16 ±.01 $kg \cdot d^{-1}$ and C ewes .22 ±.01 $kg \cdot d^{-1}$ over the 158 d growth phase. At the conclusion of the growth phase, R ewes weighed 17% less than C ewes ($P<.0001$) (figure 3), as compared to the 25% difference in body weights reported at the conclusion of the restriction period.

Control ewes on ad libitum intakes of alfalfa pellets did not gain as rapidly as expected during this trial. Unpublished data from the Michigan State University Sheep Teaching and Research Center reported ADG of .35 $kg \cdot d^{-1}$ (Shane, 1994) in wether lambs fed ad libitum intakes of alfalfa pellets. While given similar genotype and body weight, wether lambs gain more efficiently than ewe lambs on equal intakes and feed

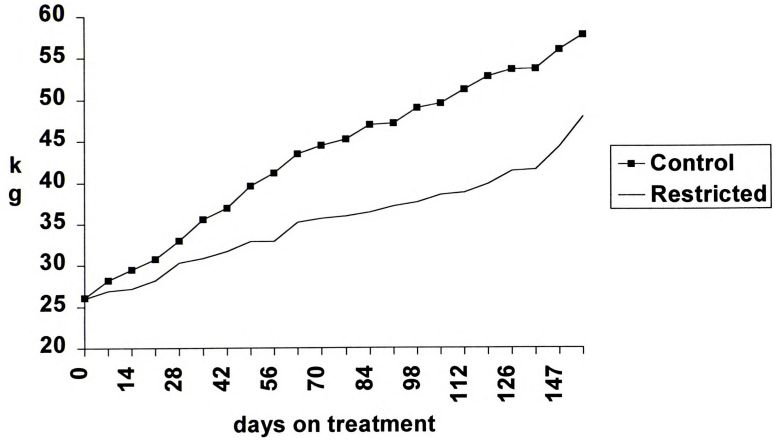

Figure 3: Ewe body weights during the 158 d growth phase

composition (NRC, 1985), however, it was expected that ADG would be greater than .22 $kg \cdot d^{-1}$ in C ewes on this study. The alfalfa pellets fed during the present study contained 13.5% crude protein and 2.06 $Mcal \cdot kg^{-1}$ ME . Based on 2.8 $kg \cdot d^{-1}$ average daily intake by C ewes over the 158 d growth phase, C ewes consumed 378 $g \cdot d^{-1}$ of crude protein and 5.8 $Mcal \cdot d^{-1}$ ME. According to NRC (1985), medium framed, 50 kg lambs, consuming 378 $g \cdot d^{-1}$ crude protein and over 3.1 $Mcal \cdot d^{-1}$ ME should gain at least .40 $kg \cdot d^{-1}$, as opposed to the .22 $kg \cdot d^{-1}$ seen here. While the difference in ADG between ewes on the current study and predicted NRC values may be explained by genetics and environment, C ewes also gained less than similar MSU lambs fed alfalfa pellets on other studies. This suggests that ewes on the current study consumed lower amounts of feed than those on other studies.

Growth Hormone. Mean serum GH concentration over a 6 h period at the conclusion of the 120 d restriction is shown in figure 4. Growth hormone in serum was greater in R ewes, 3.3 ± .14 $ng \cdot mL^{-1}$ min than C ewes, 2.7 ± .13 $ng \cdot mL^{-1}$ min *(P<.0005)*, when expressed as log transformed, mean area under the curve. These results agree with previous work which suggests that in pre-pubertal sheep (McFadden et al., 1990; Johnsson et al., 1985) and cattle (Sejrsen, 1981), a decreased plane of nutrition increased concentration of GH in serum or plasma. Johnsson et al., (1985) measured mean concentration of plasma GH in 18 week-old crossbred ewes fed a 95:05

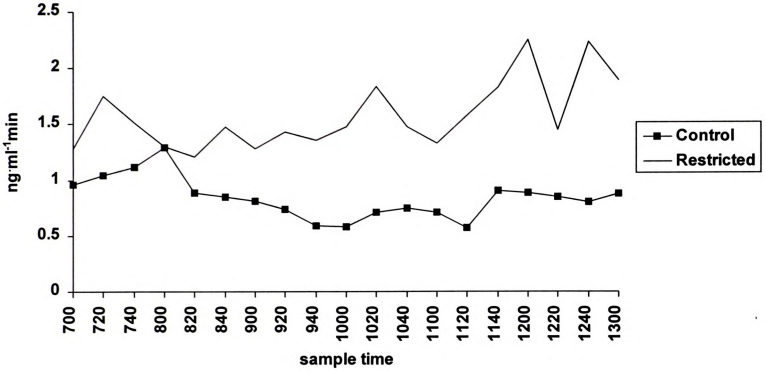

Figure 4: Six-hour growth hormone in serum on d 120 of a pre-pubertal dietary Restriction period in ewes.

concentrate pellet : grass hay diet to grow at high (H) 220 $g \cdot d^{-1}$ or low (L), 110 $g \cdot d^{-1}$ rates of gain, and reported plasma GH concentrations of 1.20 and 1.5 $ng \cdot ml^{-1}$ for H and L ewes respectively. Mc Fadden et al. (1990) reported serum GH concentrations of 5.5 and 8.0 $ng \cdot mL^{-1}$ in crossbred ewe lambs fed at ad libitum (A) or restricted (R) intakes of a concentrate diet to gain 240 and 120 $g \cdot d^{-1}$ respectively, from 7 to 22 weeks of age. Again, GH concentration was increased in ewes on low levels of intake. Unlike the Johnsson et al. (1985) study or the present study which measured growth hormone in blood samples taken over 28 and 6 h periods, respectively, McFadden et al. (1990) measured serum growth hormone in samples taken twice daily (0800 and 1600), one day a week for 9 weeks. By taking samples at the beginning and end of a time period rather than sampling over the course of time, McFadden et al. (1990) fail to take into account the circadian variation in growth hormone concentration, which may account for the larger serum GH concentrations than those reported in the present trial. Serum GH concentrations reported by Johnsson et al. (1985) and McFadden et al.(1990) are numerically different from those reported in the current trial, however the pattern of variation in growth hormone concentration is consistent with the pattern seen in the present trial; ewes on restricted pre-pubertal intakes had increased growth hormone concentration.

Breeding. At the conclusion of the growth phase, estrus was synchronized with the insertion and later removal of half of a progestin implant, and all ewes were exposed for one 20 d period of natural service breeding. Based on data provided by ultra-sound at 90 days after exposure to the rams, 30 of 51 ewes were pregnant, representing a pregnancy rate of 58.8%. In general, pregnancy rates in ewe lambs are highly variable, ranging from 30 to 90% depending on such factors as season, breed and level of nutrition. More C ewes (69.2%) were identified as pregnant at 90 days post-breeding than R ewes (44%) ($P<.01$) (table 7). While onset of puberty was not measured in the present study, the difference in pregnancy rate between treatments suggests that the onset of puberty may have been delayed in R ewes. McCann et al. (1989) determined that ewes fed a 40:60 Bermuda grass:concentrate mix to gain 239 $g \cdot d^{-1}$ from weaning to onset of puberty reached puberty at a younger age than ewes fed a 60:40 Bermuda grass:concentrate mix to gain 179 $g \cdot d^{-1}$ during the same period. This may explain the lower pregnancy rates in R ewes in the present study. Ewes on the present study were exposed to rams for one 20 d period of natural service breeding, whereas ewes under typical management schemes would usually be provided a longer breeding season. Regardless of treatment, pregnancy rates on the current trial may have been improved had ewes been exposed to rams for a longer period of time.

Table 7: A summary of pregnancy and lambing rates of Control and Restricted ewes.

Treatment	No. pregnant	Pregnancy Rate (%)		No. Lambing	No. Twins	Twinning Rate (%)	No. Singles	Single rate (%)	
Control (n=26)	18	69.2	P<.01	15	8	53	7	46.7	P<.05
Restricted (n= 25)	11	44		11	1	9	8	88.9	
Total	29	56.9		24	9	38	15	62.5	

Three C and two R ewes aborted prior to the expected parturition date, for unknown reasons and one C ewe identified by ultra-sound as pregnant was not. As a result, 15 C ewes and 9 R ewes lambed, with C ewes having 8 sets of twins and R ewes only one set of twins (table 7) ($P<.05$). The large treatment difference in sets of twins produced was not expected, since R ewes basically underwent a period of flushing prior to breeding. Flushing is defined as placing females on a gaining level of nutrition before breeding to stimulate greater rates of ovulation (Taylor, 1995). It is possible that no improvement was seen in the ovulation rates of R ewes due to flushing because they were not fully mature during the 38 d realimentation period or at breeding and hence the additional feed was utilized for growth and not to improve reproductive performance.

The larger number of twins produced by C ewes may be explained by the difference in body weight between C and R ewes at breeding. Control ewes weighed 57.7 kg and R ewes 47.9 kg when final growth phase weights were taken 22 days prior to breeding. If C and R ewes continued to gain at their respective rates of .17 and .25 kg $\cdot$ d^{-1}, C ewes weighed approximately 61.4 kg and R ewes 57.7 kg at breeding. Ovulation rates and hence incidence of twinning, may have been lower in R ewes as a result of a difference in live-weight at the time of breeding. Smith (1988) cites a number of studies which support the theory that in ewes, there is a 2% increase in ovulation rate for each

additional kg of live-weight immediately prior to mating.

Milk Production. Milk production was measured twice weekly over a 60 d lactation. Treatment, breed of ewesire and type of rearing effects were included in the model to analyze milk production. There was no difference in first lactation milk yield between ewes fed alfalfa pellets to gain .11 $kg \cdot d^{-1}$ prior to puberty (R) and ewes allowed ad libitum intakes of alfalfa pellets to gain .22 $kg \cdot d^{-1}$ (C) during the same period (P>.10) (figure 5). Control ewes produced an average of 2.06 $kg \cdot d^{-1}$ over the 60 d lactation, while R ewes produced 2.14 $kg \cdot d^{-1}$.

Control ewes gained .22 $kg \cdot d^{-1}$ and R ewes .11 $kg \cdot d^{-1}$ over the 120 d restriction period; a difference of 50%. It is possible that at .22 $kg \cdot d^{-1}$ ADG, C ewes were not gaining at a great enough rate to impair mammary development. Johnsson et al. (1985), saw a greater amount of parenchyma in ewe lambs fed a 95:05 concentrate pellet : grass hay diet (3.32 $Mcal \cdot kg^{-1}$ ME, 187 g $CP \cdot d^{-1}$) to gain .11 $kg \cdot d^{-1}$ than in those fed the same diet to gain .22 $kg \cdot d^{-1}$, suggesting that diet composition may be more important than ADG in altering mammary composition. VandeHaar et al. (1997) suggest that the ratio of protein to energy may play a role in the development of the mammary gland in dairy heifers, and that if sufficient protein is available to the animal, mammary gland development will not be impaired regardless of ADG. Capuco et al. (1995) found lower amounts of parenchyma in pre-pubertal heifers fed a corn silage diet to gain .93

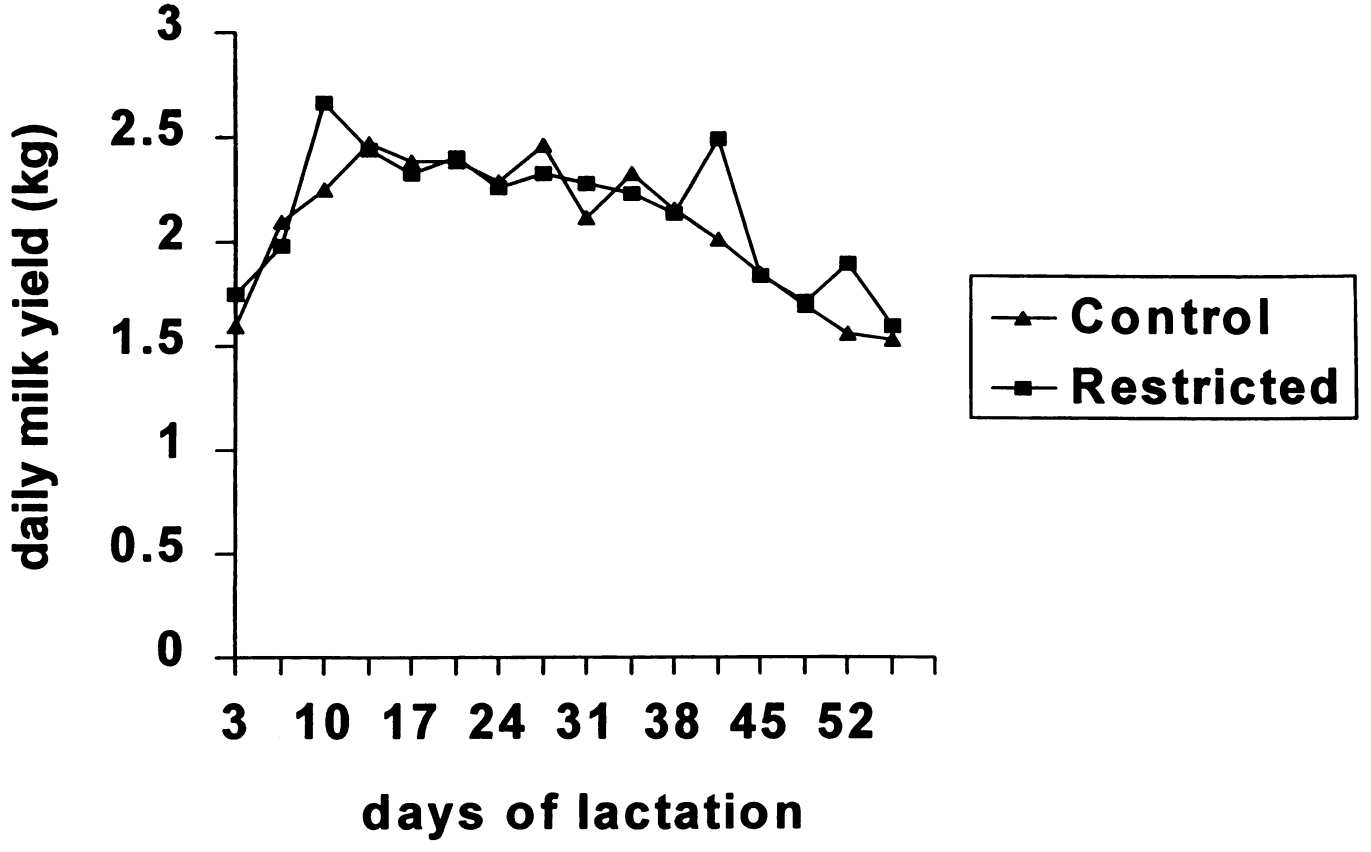

Figure 5: Daily milk production, C vs. R ewes

$kg \cdot d^{-1}$ than in those fed alfalfa silage to gain the same amount. The corn silage diet on the Capuco et al. (1995) trial contained 3.4 $Mcal \cdot kg^{-1}$ ME and 16% crude protein, and the alfalfa silage diet, while similar in ME at 3.1 $Mcal \cdot kg^{-1}$ contained more crude protein at 22%. In the present study, crude protein was not limiting in either C or R ewes during the restriction period and no difference was seen in milk production. Based on this information, it is possible that if a diet is high in crude protein, an animal may be fed for rapid gain without impairing mammary development. Additional research in this area is warranted.

The lack of difference in daily milk yield between C and R ewes in this trial suggests that restricting pre-pubertal intake did not increase mammary parenchyma in a manner that would increase first lactation milk yield. It is possible that mammary parenchyma was increased in R ewes on the current trial, but that a treatment difference in milk production was not detected due to the low number of ewes that were actually milked. With only 24 lactating ewes, it is possible that there was insufficient power to detect a difference in milk production.

Ewes nursing twins generally produce more milk than ewes nursing singles, however, this study showed no difference in milk yield based on type of birth ($P>.10$), or the interaction of treatment by type of birth, which was not expected (figure 6). Henry and Benson, (1990) and Gardner and Hogue (1964)

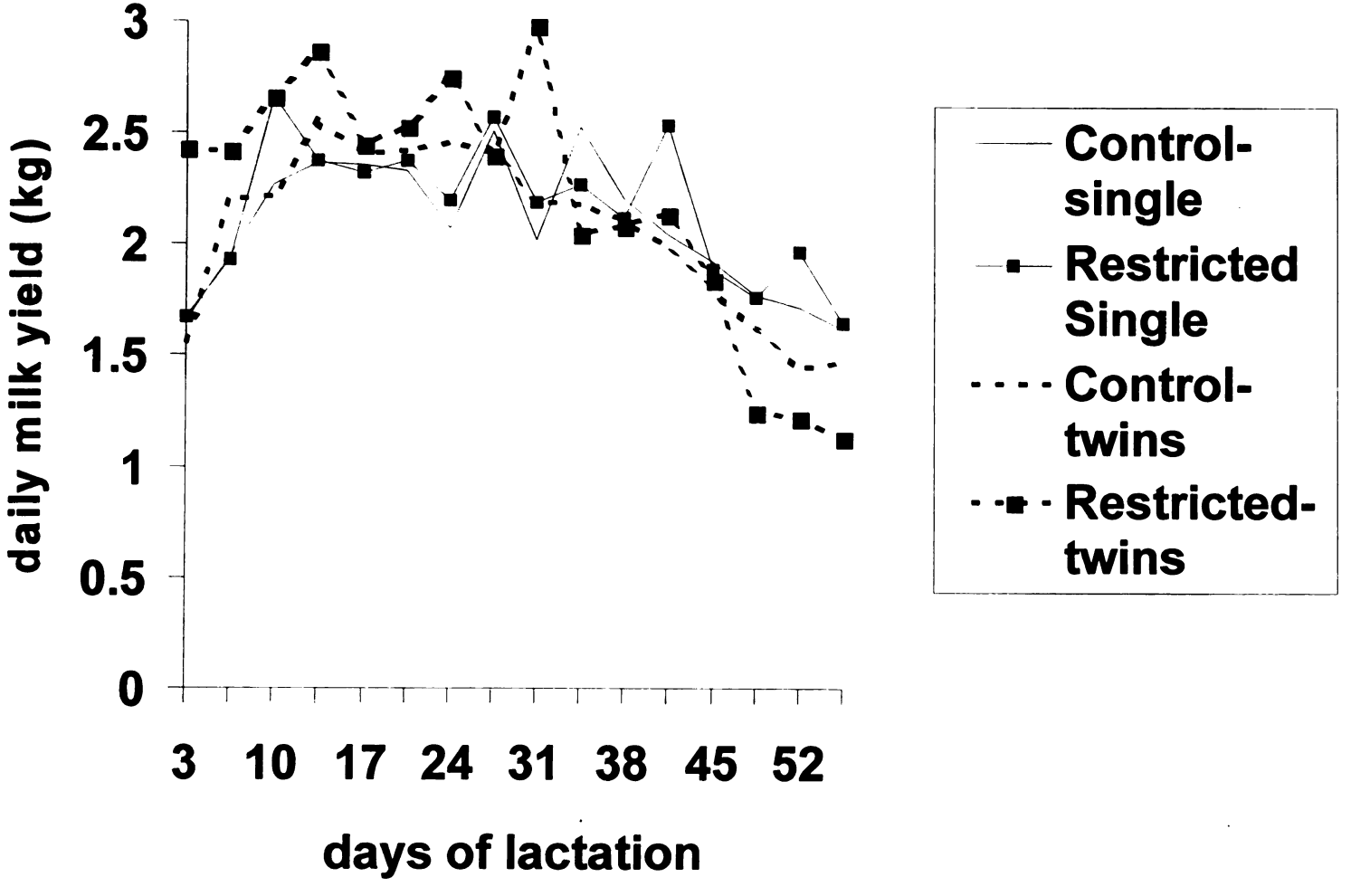

Figure 6: Daily milk yield (kg), control vs. restricted ewes, twins vs. singles

reported that ewes suckling twin lambs produced more milk than ewes nursing single lambs. The NRC (1985) suggests that a ewe nursing twin lambs produces 20 to 40% more milk than a ewe nursing one lamb. Control ewes on the present trial had 8 sets of twins and 7 singles, while R ewes had one set of twins and 8 singles. The lack of difference in milk yield between ewes nursing twins and singles is likely due to a lack of sufficient animal numbers to detect a difference.

Suffolk sired ewes in this study had greater daily milk production than Rambouillet sired ewes, 2.37 $kg \cdot d^{-1}$ and 1.85 $kg \cdot d^{-1}$ respectively. This is in agreement with Sakul and Boylan (1992) who found that Suffolk ewes produced more milk daily; 680 mL than Finnsheep; 526 mL, Lincoln; 487 mL, Romanov; 299 mL and Targhee; 591 mL ewes.

The lactation curves of cattle and sheep follow a similar pattern over time in that milk yield increases rapidly in the days immediately following parturition, then peaks and subsequently declines (Taylor, 1995). Based on this information, one would expect that milk production measurements at two successive points would be more highly correlated than measurements that were not successive, which was the case on the present trial (appendix A). Using Proc Mixed and SAS® (6.11) it was determined that milk production on the first and second days of measurement were more highly correlated (r=.71) than

measurements taken on the first and third days of measurement (r=.50) and that the correlations declined as measurement points became further apart.

McCann et al. (1989) reported daily milk production ranging from 1.7-1.9 $kg \cdot d^{-1}$ in ewe lambs measured at 25 days of lactation. Umberger et al. (1985) reported milk yields ranging from 1.3-1.6 $kg \cdot d^{-1}$ at 20, 40 and 60 days of lactation. The 60 d average milk yield values reported in this trial; C; 2.06 $kg \cdot d^{-1}$ and R; 2.14 $kg \cdot d^{-1}$, are higher than those reported by McCann et al. (1989) and Umberger et al. (1985), which may be a result of the sampling procedures used. Ewes on the current trial were milked at 16 points over the course of a 60 day lactation and thus give a better representation of milk yield across lactation, unlike the McCann et al. (1989) and Umberger et al. (1985) studies, which measure milk at only one and three points respectively.

Milk Composition. There was a trend for greater milk dry matter (P<.10) in R ewes; 22.2 ±.77% than C ewes; 20.4 ±.57% and R ewes had greater milk fat content on a percent dry matter basis (P<.005) than C ewes; 13.1 ±.67% and 10.5 ±.45% respectively. (figures 7 and 8). There was no difference in milk crude protein. In agreement with the findings of Gardner and Hogue (1964), percent milk DM, fat and crude protein were high in early lactation, declined by the second week of lactation and steadily increased from that point until the

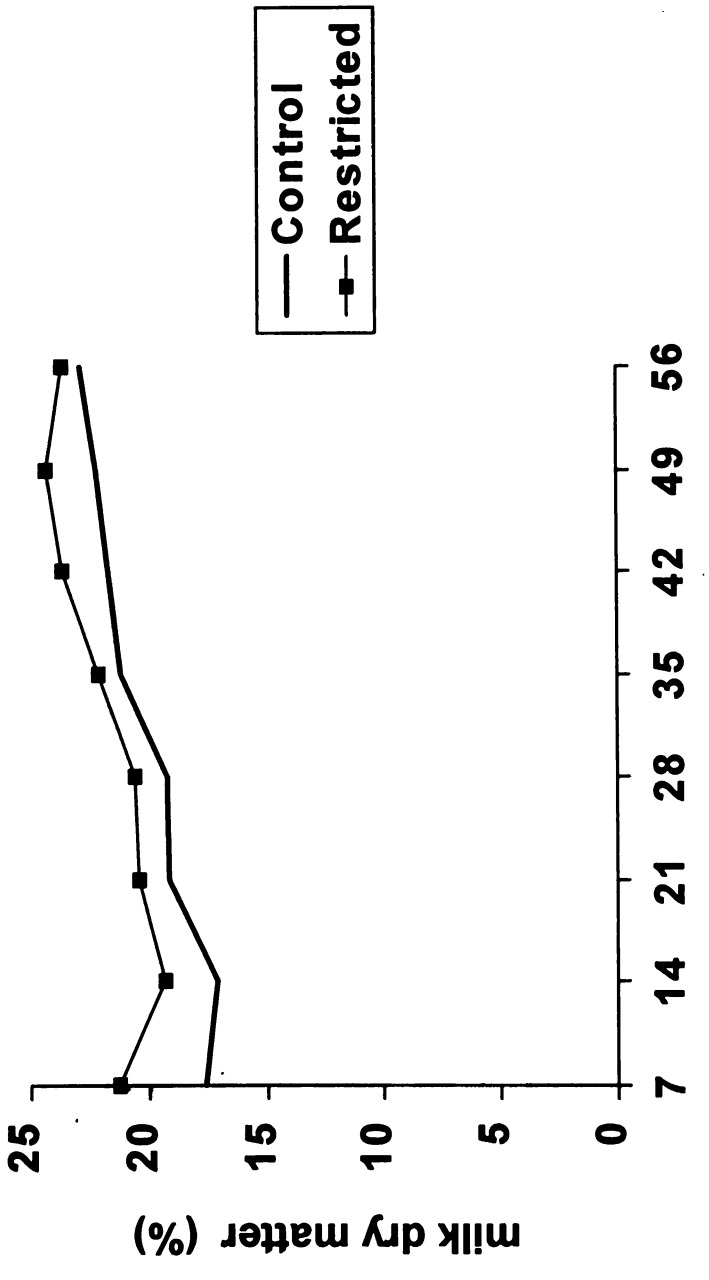

Figure 7: Milk dry matter over a 60 d lactation, C vs. R ewes

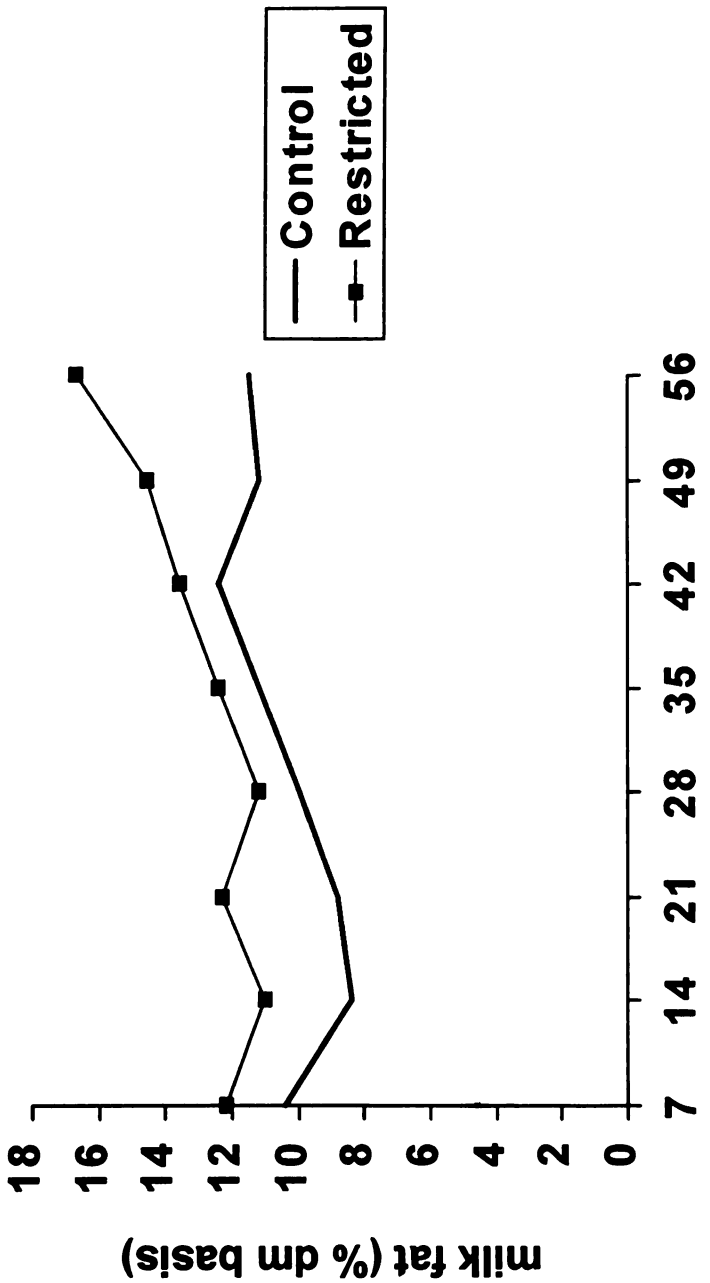

Figure 8: Milk fat over a 60 d lactation, % dry matter basis, C vs. R ewes

end of lactation, regardless of treatment (P<.0005). There were no sire breed of ewe nor rearing-type effects on milk DM, fat or crude protein.

The NRC (1985) reports that at 2.5 weeks post-partum, ewe's milk contains 18.2% DM, 5 to10% fat and 24.7% crude protein on a DM basis. The milk DM and fat composition reported in the current study are higher than NRC values ; 20.4-22.2% DM, 10.5-13.1% fat, while crude protein is slightly lower; 20.1-21.1%. Milk composition was determined at 8 points of lactation on the current trial. If values reported in the NRC (1985) were calculated at fewer than eight points of lactation, it is possible that the values determined in the current trial are more representative than NRC (1985) values for DM, fat and crude protein of ewe's milk. Ewe's milk composition may also vary by breed (Sakul and Boylan, 1992) and level of nutrition (Gardner and Hogue, 1964), which also may contribute to the differences between milk composition values reported in the current trial and those reported in the NRC (1985). Sakul and Boylan (1992) found that Suffolk ewes produced milk of greater fat content, 6.6%, than Finnsheep, 6.0%, Romanov, 5.9%, and Targhee , 5.9%, ewes. Dorset ewes produced milk of greater protein content, 6.1%, than Finnsheep, 5.4%, Lincoln, 5.6% and Targhee; 5.7%, ewes. Gardner and Hogue (1964) determined that ewes fed at 94% of NRC requirements for DE produced milk of lower fat content, 6.4% than ewes fed at 111% of NRC requirements for DE, although milk crude protein did not change by level of nutrition.

Restricted ewes produced more milk as a percent of body weight, 3.14 and 2.6% in R and C ewes, respectively (P<.05), with a higher percent milk fat than C ewes. Restricted ewes weighed less than C ewes during the 60 d lactation phase, (P<.05) averaging 70.4 kg and 77.5 kg, respectively, and ewe weight did not change significantly over the course of lactation, nor was it affected by type of rearing. Restricted and C ewes were housed together during lactation and were offered 3.5 $kg \cdot d^{-1}$ alfalfa hay and 1 $kg \cdot d^{-1}$ of a concentrate mix (tables 4 and 5) if nursing twins and 3.5 $kg \cdot d^{-1}$ alfalfa hay and .9 $kg \cdot d^{-1}$ of the same mix if nursing singles. It is possible that given the difference in body weight, R ewes consumed the same amount of feed as C ewes, met their maintenance requirements and had enough Net energy remaining to increase both milk production as a percent of body weight and milk fat over levels produced by the heavier C ewes.

Lamb Growth. There was no difference in lamb weight by treatment of ewe (P>.10). Lambs reared as singles were heavier than lambs reared as twins, concluding the 60 d lactation period at 24 and 18 kg of body weight respectively (P<.0005) (figure 9). Single lambs gained .29 $kg \cdot d^{-1}$ and twin lambs gained .23 $kg \cdot d^{-1}$ regardless of treatment. Heavier single lambs are to be expected in that a single lamb consuming a quantity of milk would be heavier than twin lambs sharing the same amount of milk of equal composition. Lambs were creep-fed for the last 30 d of the trial and lamb weight increased over time.

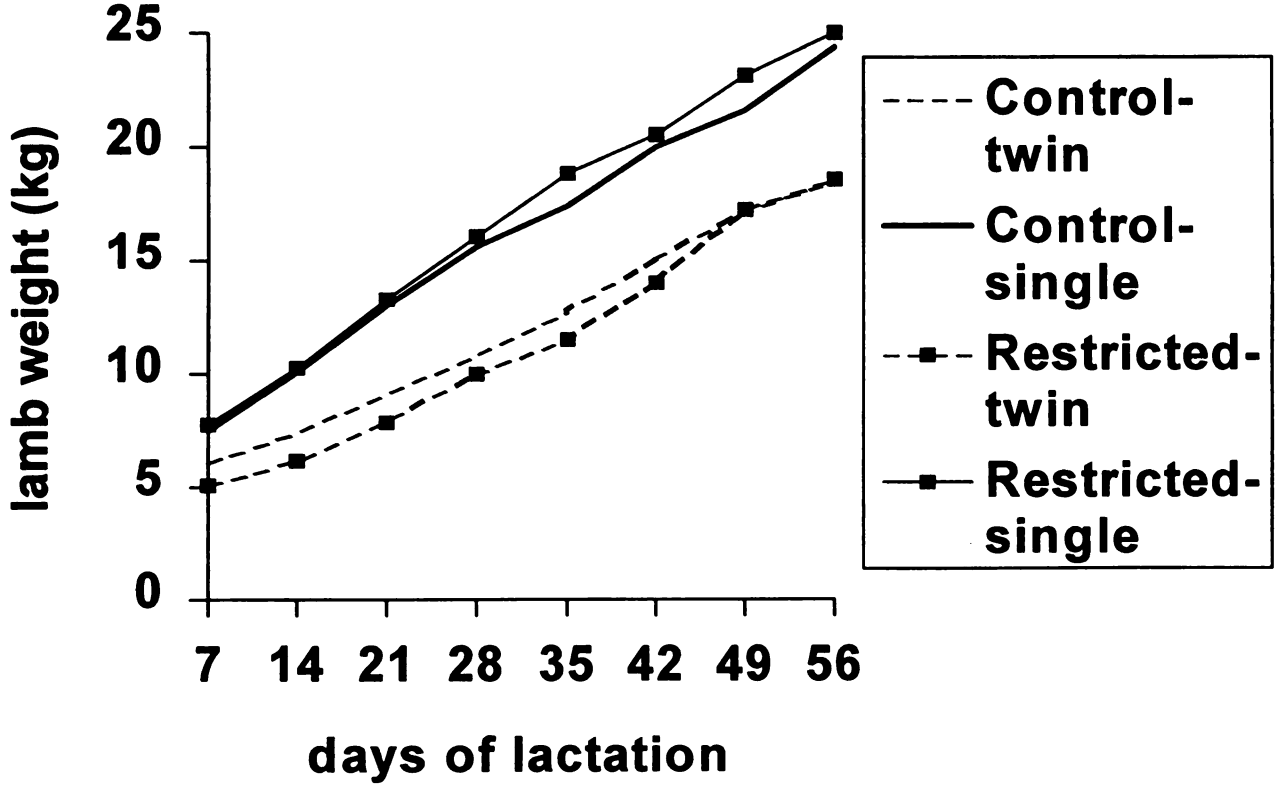

Figure 9: Body weights of lambs reared by C or R ewes

Chapter Five

COMPUTED TOMOGRAPHY AS A METHOD OF DETERMINING MAMMARY AND PARENCHYMAL VOLUME IN THE LIVE EWE

Introduction

Mammary gland composition in cattle, sheep and goats is often measured through the post-mortem removal of the udder and it's subsequent chemical analysis (Sejrsen, 1981; Mc Cann et al.; 1989, Bowden et al., 1995). Wet trimmed and untrimmed mammary weight, dry fat free tissue (DFFT), mammary protein and mammary DNA and RNA are components that have been quantified using mammary dissection and chemical analysis. In some studies, parenchyma and stroma are separated and mammary parenchymal weight, DFFT, protein and RNA have been calculated (Johnsson and Hart, 1985; Anderson, 1975).

Mammary dissection and chemical analysis have provided much information regarding the composition of tissues that form the mammary gland, however, there are two primary drawbacks to the use of this method. It is not possible to correlate mammary parenchymal cell number to future milk production in the same animal, given the terminal nature of the method. In addition, the determination and separation of parenchymal tissue and stroma is based on visual characteristics, making it a highly subjective process.

Computed Tomography (CT) is a non-terminal method which may allow for the successful measurement of mammary composition in vivo, while potentially reducing the variation resulting from human error with respect to tissue identification. The CT process involves image reconstruction from emission or transmission measurements collected from the subject. This information is processed by a computer which uses mathematical techniques to build sectional images of internal anatomy. Computed Tomography reports attenuation values for each sectional image based on variation in tissue density and these attenuation values or CT numbers are expressed in Houndsfield units (HU) (Seeram, 1994). The use of CT allows for the separation of tissues which differ in density by as little as 1% (GE Medical Systems, 1995). Reported CT values for water, air and bone are 0, -1,000 and 1,000 respectively.

Sorensen et al., (1987) quantified mammary parenchymal volume and weight of twenty-five dairy heifers using CT and chemical analysis of dissected glands. They determined that the correlation coefficients between measurements of parenchymal tissue by dissection and CT were r^2=.80 for fat free parenchyma, .78 for parenchymal protein, and .62 for total parenchyma.

Glaser et al., (1991) used CT to compare mammary parenchymal volume in 35 Angus X Holstein heifers as determined by CT and chemical analysis of dissected glands, using a technique similar to that described by Sorensen et al., (1987). Glaser et al. (1991) concluded that CT was an effective method to

quantify total mammary volume and parenchymal tissue volume based on comparison to parenchymal tissue weight determined by dissection, however, correlation coefficients were not reported.

Estimates of total mammary and parenchymal volume provided by CT of dissected mammary glands correlate highly with the chemical analysis of those glands (Sorensen et al, 1987). Work with CT to date has been conducted with the dissected mammary glands of cattle, given the limited size of the CT gantry, or chamber in which subjects are placed to provide axial images. Consequently the correlation between in vivo mammary gland composition and future milk production has yet to be addressed. Given their smaller size, it may be possible to use CT to determine Total mammary and parenchymal volume in ewes and subsequently correlate composition data with future milk production. The objective of his study was to evaluate a method by which CT could be used to determine mammary and parenchymal volume in the live ewe.

Materials and Methods. Ten Dorset X Suffolk X Rambouillet crossbred ewes ranging from 81 to 100 kg and varying in age and parity were selected for mammary gland CT scanning (table 8). Ewes had weaned their final lamb crops 2 to 6 months prior to CT scanning and were anesthetized using 3 to 5% Halothane with an oxygen flow greater than 5 mL $\cdot$ lb^{-1}. Percent inhalant was adjusted to maintain depth of anesthesia at a level such that movement of the ewe was inhibited. Ewes were placed on their backs and restrained on the CT

Table 8: A summary of ewe age, parity and weaning date of final lamb crop

Ewe ID	age (yr)	No. lamb crops	weaning date of final lamb crop	date of CT scan
102	5	3	June 95	Dec 95
99	5	3	June 95	Dec 95
94	4	3	June 95	Nov 95
323	3	2	June 95	Nov 95
411	3	2	June 95	Aug 95
401	3	2	June 95	Dec 95
28	9	9	June 95	Nov 95
21	6	5	June 95	Nov 95
255	3	2	June 95	Aug 95
260	3	3	June 95	Dec 95

(CT 9800, GE Medical Systems) table. In addition, hind legs were secured to the table using a small section of duct tape to prevent damage to the machine should anesthesia wear off during the procedure. The table was adjusted such that the first axial images taken were immediately anterior to the mammary gland. The CT table then moved through the gantry, with an axial image recorded every 3 mm from the anterior to the posterior border of the mammary gland.

Once axial images were recorded by the computer, each image of the mammary gland was traced using the TRACE function of the CT9800. Total gland area (cm^2) for each 3 mm thick axial image was determined using the REGION OF INTEREST function and values were recorded. The range of CT numbers assigned to parenchymal tissue, 40-100 HU, was calculated by determining the CT numbers in areas of the gland where parenchyma is normally found, based on dissection of glands. Area of parenchymal tissue (cm^2) in the traced mammary gland of each 3 mm thick axial image was then determined using the DENSITY MASK function found in the ANALYSIS MENU of the CT 9800. Total mammary volume (cm^3) was determined by adding the total area (cm^2) for all axial images of a ewe and multiplying the value by .3 cm. Mammary parenchymal volume was calculated in the same manner.

Results and Discussion. Total mammary gland volume as determined by CT ranged from 121.5 cm^3 to 643.6 cm^3 and total parenchymal volume ranged

from 38.4 cm^3 to 138.8 cm^3. The percent of gland volume occupied by parenchymal volume ranged from 6.6% to 72% (table 9). McCann et al (1989), reported a half mammary gland total area of 159.4 cm^2 in glands that were dissected from ewes, infused with a vinyl chloride solution, sliced into 4 mm thick sections, photographed and measured with a planimeter. When 159.4 cm^2 is multiplied by .4 cm to determine half mammary gland total volume, a value of 63.8 cm^3 is obtained. When this value is doubled to estimate total mammary gland volume, the resultant value of 127.6 cm^3 falls within the range of total mammary volume determined by CT on the current project. While the total mammary volume calculated for the McCann et al (1989) ewes is on the lower end of the range reported here, the McCann et al. (1989) ewes were 7 months of age and were nulliparous, hence the mammary glands probably had not matured to the level of the ewes on the current trial. Clearly the variation between CT scanned ewes in total mammary gland and parenchymal volume is large. This variation can likely be accounted for by the differences in size, age and parity among the ewes studied. An additional study collecting data from ewes of similar size, age and parity is warranted.

Sorensen et al. (1987) reported total mammary volume of 1231 cm^3 and total parenchymal volume of 145 cm^3 in dissected, CT scanned mammary glands of 25, 260 kg yearling dairy heifers. Based on these data, percent parenchymal volume of these heifers was 11.8%. Given the size difference between sheep

Table 9: A summary of total mammary gland and parenchymal area and volume as determined by CT scan of 10 live ewes.

Ewe ID	Total gland area (cm^2)	Total parenchymal area (cm^2)	Total gland volume (cm^3)	Total parenchymal volume (cm^3)	Percent parenchymal volume
102	2131.2	402.3	643.6	120.7	18.8
99	1519.2	462.6	455.7	138.8	30.4
323	719.2	336.7	215.8	101	46.8
401	404.9	291.4	121.5	87.4	72
94	1084.9	242.6	325.5	72.8	22.3
255	607.6	266.7	182.3	80.0	43.9
21	581.5	343.3	174.5	103.00	59.0
411	795.5	262.0	238.6	78.6	32.9
260	1934.3	128.1	580.3	38.4	6.6
28	1325.18	366.3	397.6	109.9	27

and cattle, the total parenchymal volume of 145 cm^3 determined by Sorensen et al. (1987) seems low when compared to corresponding values for the ewes on the present study, which ranged from 38.4 to 138 cm^3. Based on size of the animal, one would expect a larger volume of parenchyma in dairy cattle, however, heifers on the Sorensen et al. (1987) study were nulliparous yearlings and had not experienced the mammary development that occurs during gestation. According to Schmidt (1971), recurring pregnancies increase the amount of mammary gland growth in dairy cattle until a mature size is reached in the third or fourth lactation, based on the maximum milk production that occurs at this time. Ewes on the present study had at least two lamb crops each and had parenchymal volumes similar to the Sorensen et al. (1987) heifers which had yet to calve. If the Sorensen et al . (1987) heifers had been through three gestation periods, the total parenchymal volume would might have been larger than 145 cm^3.

There was a large variation in percent parenchymal volume in the ewes on the present study (figure 10). Percent parenchymal volume was calculated by dividing total parenchymal volume by total gland volume and multiplying by 100. Depending on the physiological state of the ewe, percent parenchymal volume calculated in this manner could change based on changes in the volume of the total mammary gland. The total mammary gland volume of a mature ewe would be larger immediately following parturition than in early gestation due to the accumulation of milk in the gland, which would suggest a lower percent mammary parenchyma, even if the parenchymal volume

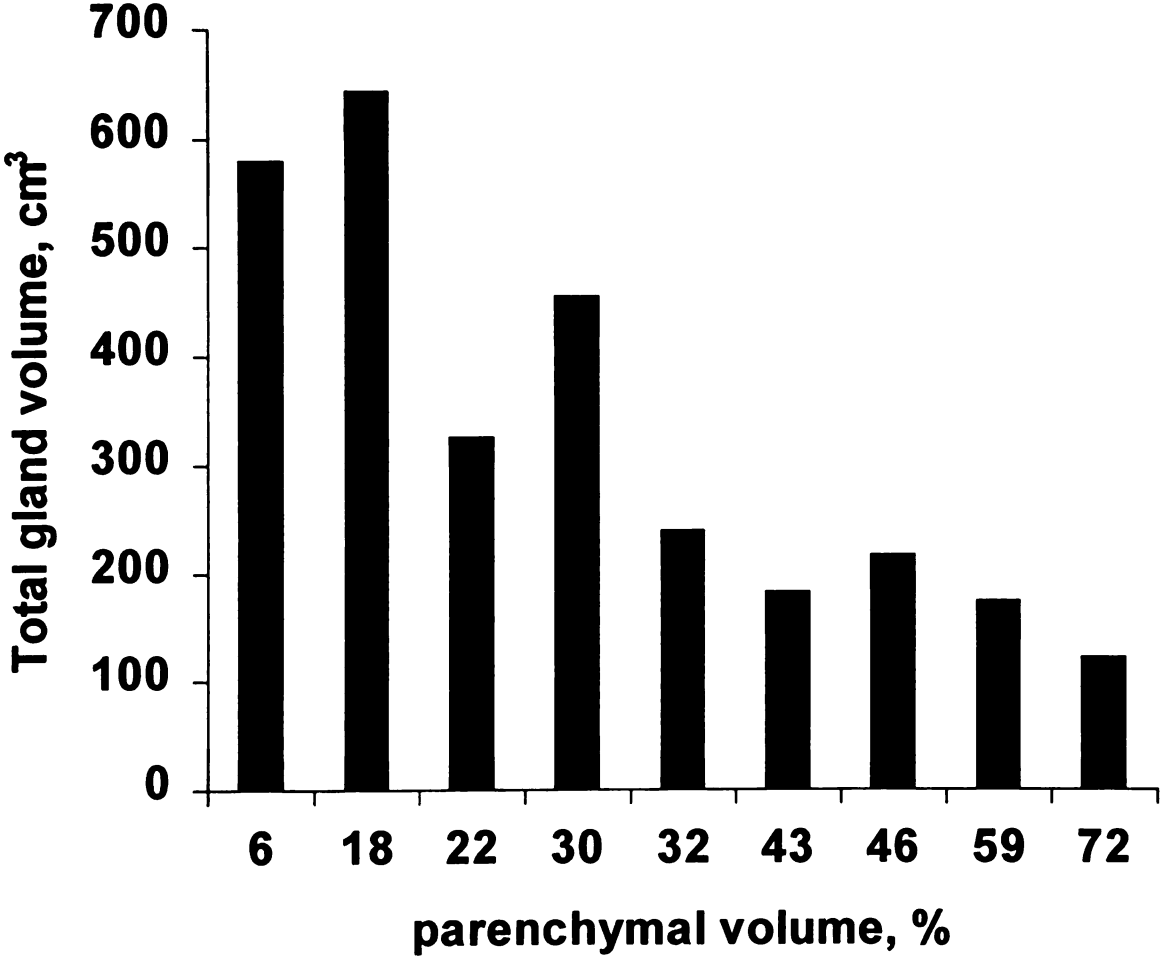

Figure 10: Total mammary gland volume vs. percent parenchymal volume

did not change significantly from one stage to the next. A more useful comparison of mammary parenchymal volume would likely come from comparing actual values as opposed to percentages.

In the first 2 ewes scanned, axial images began at a point more anterior to the mammary gland than necessary. Consequently, a decision was made as to which image represented the beginning of the mammary gland. In this respect, the CT process was subjective, however, the decision made as to where the mammary gland started is similar to that made when deciding at what point to dissect a mammary gland. So long as every attempt is made to get as much of the gland as possible on each ewe, it is not likely that such a decision has a major effect on mammary gland measurement.

By choosing 40 to 100 H.U. as the range of attenuation values representing parenchyma, it is possible that some parenchymal tissue may have been excluded from measurement. Sorensen et al. (1987), however, determined that correlations of CT estimates with estimates of parenchyma by dissection were nearly identical when lower limits were set at 0, 20 and 40 H.U.; .81, .79, and .78, respectively. It is likely that the values chosen in this study were appropriate, however, an additional study to determine the most appropriate range of attenuation numbers representing parenchyma in the live ewe may be warranted.

Conclusions. A method by which CT could be used to determine mammary and parenchymal volume in the live ewe, was studied. The groundwork has been set for

future studies, which would allow the correlation of parenchymal cell volume and milk production in the same ewe. In addition, mammary glands could be CT scanned for composition at different stages of development or at different phases of a project without sacrificing animals and statistical power.

SUMMARY AND CONCLUSIONS

The objectives of the experiments described in this thesis were: 1) to determine the effect of a pre-pubertal dietary restriction and subsequent realimentation on future milk production of the replacement ewe; 2) to compare growth hormone profiles of restricted and ad libitum fed pre-pubertal ewes; and 3) to evaluate a method by which Computed Tomography (CT) could be used to determine mammary and parenchymal volume in the live ewe.

Dietary treatments were imposed on ewe lambs over a 158 d growth phase consisting of a 120 d restriction and a 38 d realimentation period. Control ewes fed alfalfa pellets at ad libitum levels of intake gained .22 $kg \cdot d^{-1}$ over the course of a 158 d growth phase. Restricted ewes consumed 1.2 $kg \cdot d^{-1}$ alfalfa pellets and gained .11 $kg \cdot d^{-1}$ during the restriction period. Restricted ewes consumed 2.7 $kg \cdot d^{-1}$ alfalfa pellets gained.22 $kg \cdot d^{-1}$ during the 38 d realimentation period and weighed less than C ewes at the conclusion of both the restriction and realimentation period. Restricted ewes consumed less feed with greater ADG than C ewes during the realimentation period , providing support to the theory that animals on a compensatory growth feeding regimen exhibit greater efficiency of gain. Compensatory growth did not provide enough gain to bring R ewes to body weights equal those of C ewes, however, and C ewes weighed more than R ewes at the conclusion of the Growth phase. As expected, R ewes had increased serum growth hormone concentration compared to C ewes at the conclusion of the 120 d restriction period, indicating that dietary treatment differences had been

of the 120 d restriction period, indicating that dietary treatment differences had been achieved during the 120 d restriction period. At the conclusion of the 158 d growth phase, estrus was synchronized and ewes were exposed to rams for one 20 d period of natural service breeding. Restricted ewes had lower pregnancy rates than C ewes , which may indicate that puberty was delayed in R ewes, although onset of puberty was not determined. At parturition, R ewes had fewer total lambs and sets of twins suggesting that ovulation rates may have been lower in R ewes. There was no difference in milk yield between R and C ewes, suggesting that the pre-pubertal dietary restriction did not increase the number of parenchymal cells in the mammary gland, or that the a difference was produced but did not remain during lactation. Restricted ewes produced more milk as a percent of body weight, and produced milk of higher fat content than C ewes. Restricted ewes weighed less than C ewes during lactation, and were offered equal amounts of feed, suggesting that R ewes consumed enough ME and crude protein to attain maintenance requirements and produce more milk as a percent of body weight, of greater fat content than C ewes. There was no difference in lamb gain by treatment, however, lambs reared as singles weighed more than lambs reared as twins.

A method by which CT could be used to determine mammary and parenchymal volume in the live ewe was evaluated, using 10 Dorset X Suffolk X Rambouillet ewes. Future studies using CT may allow for the determination of mammary composition at

various stages of development without sacrificing animals or statistical power. In addition, the correlation of parenchymal cell volume and milk production in the same ewe may be determined.

Restricting pre-pubertal dietary intakes of ewe lambs as compared to ad libitum fed Controls increased serum growth hormone concentration, but did not increase first lactation milk yield. Restricted ewes produced more milk as a percent of body weight, with greater percent milk fat than ad libitum fed Controls, however, there was no difference in lamb gain by treatment of ewe.

APPENDIX

APPENDIX A

R Correlation Matrix for EWE(TRT) 433 C

Row	COL1	COL2	COL3	COL4	COL5	COL6
1	1.00000000	0.70966392	0.50362288	0.35740299	0.25363601	0.17999632
2	0.70966392	1.00000000	0.70966392	0.50362288	0.35740299	0.25363601
3	0.50362288	0.70966392	1.00000000	0.70966392	0.50362288	0.35740299
4	0.35740299	0.50362288	0.70966392	1.00000000	0.70966392	0.50362288
5	0.25363601	0.35740299	0.50362288	0.70966392	1.00000000	0.70966392
6	0.17999632	0.25363601	0.35740299	0.50362288	0.70966392	1.00000000
7	0.12773690	0.17999632	0.25363601	0.35740299	0.50362288	0.70966392
8	0.09065027	0.12773690	0.17999632	0.25363601	0.35740299	0.50362288
9	0.06433122	0.09065027	0.12773690	0.17999632	0.25363601	0.35740299
10	0.04565355	0.06433122	0.09065027	0.12773690	0.17999632	0.25363601
11	0.03239868	0.04565355	0.06433122	0.09065027	0.12773690	0.17999632
12	0.02299217	0.03239868	0.04565355	0.06433122	0.09065027	0.12773690
13	0.01631671	0.02299217	0.03239868	0.04565355	0.06433122	0.09065027
14	0.01157938	0.01631671	0.02299217	0.03239868	0.04565355	0.06433122
15	0.00821747	0.01157938	0.01631671	0.02299217	0.03239868	0.04565355
16	0.00583164	0.00821747	0.01157938	0.01631671	0.02299217	0.03239868

COL7	COL8	COL9	COL10	COL11	COL12	COL13
0.12773690	0.09065027	0.06433122	0.04565355	0.03239868	0.02299217	0.01631671
0.17999632	0.12773690	0.09065027	0.06433122	0.04565355	0.03239868	0.02299217
0.25363601	0.17999632	0.12773690	0.09065027	0.06433122	0.04565355	0.03239868
0.35740299	0.25363601	0.17999632	0.12773690	0.09065027	0.06433122	0.04565355
0.50362288	0.35740299	0.25363601	0.17999632	0.12773690	0.09065027	0.06433122
0.70966392	0.50362288	0.35740299	0.25363601	0.17999632	0.12773690	0.09065027
1.00000000	0.70966392	0.50362288	0.35740299	0.25363601	0.17999632	0.12773690
0.70966392	1.00000000	0.70966392	0.50362288	0.35740299	0.25363601	0.17999632
0.50362288	0.70966392	1.00000000	0.70966392	0.50362288	0.35740299	0.25363601
0.35740299	0.50362288	0.70966392	1.00000000	0.70966392	0.50362288	0.35740299
0.25363601	0.35740299	0.50362288	0.70966392	1.00000000	0.70966392	0.50362288
0.17999632	0.25363601	0.35740299	0.50362288	0.70966392	1.00000000	0.70966392
0.12773690	0.17999632	0.25363601	0.35740299	0.50362288	0.70966392	1.00000000
0.09065027	0.12773690	0.17999632	0.25363601	0.35740299	0.50362288	0.70966392
0.06433122	0.09065027	0.12773690	0.17999632	0.25363601	0.35740299	0.50362288
0.04565355	0.06433122	0.09065027	0.12773690	0.17999632	0.25363601	0.35740299

COL14	COL15	COL16
0.01157938	0.00821747	0.00583164
0.01631671	0.01157938	0.00821747
0.02299217	0.01631671	0.01157938
0.03239868	0.02299217	0.01631671
0.04565355	0.03239868	0.02299217
0.06433122	0.04565355	0.03239868
0.09065027	0.06433122	0.04565355
0.12773690	0.09065027	0.06433122
0.17999632	0.12773690	0.09065027
0.25363601	0.17999632	0.12773690
0.35740299	0.25363601	0.17999632
0.50362288	0.35740299	0.25363601
0.70966392	0.50362288	0.35740299
1.00000000	0.70966392	0.50362288
0.70966392	1.00000000	0.70966392
0.50362288	0.70966392	1.00000000

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