Developmental and Nutritional Regulation of the Prepubertal Heifer Mammary Gland: I. Parenchyma and Fat Pad Mass and Composition

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ABSTRACT

Prior to puberty, elevated nutrient intake has been shown to negatively affect prepubertal mammary development in the heifer. The objective of this study was to evaluate the effects of increased nutrient intake on mammary development in Holstein heifers at multiple body weights from birth through puberty. Specifically, this study evaluated the effects of nutrient intake and body weight at harvest on 1) total weight and DNA content of the parenchyma (PAR) and mammary fat pad (MFP) and 2) PAR and MFP composition. Starting at 45 kg of body weight, heifers ($n = 78$) were assigned to either a restricted (R) or elevated (E) level of nutrient intake supporting 650 (R) or 950 (E) g/d of body weight gain. Heifers were harvested at 50-kg increments from 100 to 350 kg of body weight. Mammary fat pad weight and DNA content were greater in E- than in R-heifers. Additionally, E-heifers had a greater fraction of lipids and a smaller fraction of protein in their MFP than did R-heifers. Parenchyma weight and DNA were lower in E- than in R-heifers; however, when analyzed with age as a covariate term, treatment was no longer a significant term in the model. Level of nutrient intake had no effect on the lipid, protein, or hydroxyproline composition of the PAR. Collectively, these data demonstrate that PAR is refractory to the level of nutrient intake whereas MFP is not. Furthermore, the covariate analysis demonstrated that age at harvest, not the level of nutrient intake, was the single greatest determinant of total PAR DNA content.

Key words: heifer, mammary development, nutrition

INTRODUCTION

In the bovine, prepubertal mammary development consists of branching and elongation of the mammary

ducts into the surrounding mammary fat pad (MFP). More extensive branching, elongation, and ultimately the appearance of secretory alveolar cells occur only after conception under the direction of the pregnancy hormones. Postnatal mammary growth occurs at an allometric rate prior to puberty and returns to an isometric rate after puberty (Sinha and Tucker, 1969). Additionally, it has consistently been demonstrated that parenchymal (PAR) mass, DNA content, or both are reduced in heifers reared on an elevated level of nutrient intake during this period of prepubertal allometric mammary growth (Sejrsen et al., 1982; Petitclerc et al., 1984; Capuco et al., 1995). Typically, experiments evaluating the effect of elevated nutrient intake on prepubertal mammary gland development have assessed mammary growth (e.g., total DNA content) immediately prior to or shortly after puberty. A detailed assessment of the temporal effects of elevated nutrient intake on mammary development from birth through puberty has yet to be conducted. Additionally, aside from the effect plane of nutrition has on MFP weight, little is known about its influence on MFP composition and DNA content.

The objective of this study was to determine the impact of elevated nutrient intake on mammary development in the Holstein heifer at multiple points between birth and puberty. To achieve this objective, Holstein heifers were assigned to a restricted or elevated level of nutrient intake starting shortly after birth. The heifers were harvested in increments of 50 kg of BW, at which time mammary weight, DNA content, and the composition of PAR and MFP were assessed. These data demonstrated that the MFP responds to elevated nutrient intake by increasing the retention of lipids and DNA content. The PAR, however, appears refractory to the endocrine signals coordinating the increased lipid deposition observed in the MFP. Furthermore, most variation in the PAR DNA content between the 2 levels of nutrient intake is explained by differences in age at harvest.

MATERIALS AND METHODS

Animals and Tissue Collection

Seventy-eight Holstein heifers were purchased within 1 wk of age from dairy farms surrounding Ithaca,

Received October 7, 2005.

Accepted June 16, 2006.

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Table 1. Milk replacer and calf starter nutrient composition on a DM basis

Item	Diet ¹		
	R-milk replacer	E-milk replacer	Calf starter
Ingredient	(% of DM)		
CP	22.0	29.3	26.7
Fat	21.2	18.9	4.2
Ca	0.95	0.99	1.41
P	1.04	0.97	0.65
Gross energy, Mcal/kg	4.94	4.98	—
NDF	NA ²	NA	18.3
ADF	NA	NA	10.2

¹R-milk replacer: milk replacer offered to heifers assigned to the restricted plane of nutrition (Land O'Lakes, Inc., St. Paul, MN); E-milk replacer: milk replacer offered to heifers assigned to the elevated plane of nutrition; calf starter: textured calf starter (Agway Inc., Syracuse, NY).

²Not applicable.

New York. After a 3- to 5-d adjustment period, the calves (44.2 kg of BW, 9.9 d old) were assigned to diets designed to sustain an average daily gain (ADG) of 650 g (**R**) or 950 g (**E**). The Cornell University Animal Care and Use Committee approved all procedures used in this study.

Prior to weaning, all heifers were fed twice daily at 0630 and 1800 h. Heifers assigned to the E treatment received a diet with 29% CP, 19% fat milk replacer at 0.32 Mcal of gross energy/kg of BW^{0.75}, whereas the R-heifers received a diet with 22% CP, 21% fat milk replacer at 0.20 Mcal of gross energy/kg of BW^{0.75} (Table 1). The protein content of milk replacers was established based on predicted requirements from the Dairy NRC calf model (National Research Council, 2001) for the targeted growth rates. Preweaned heifers were weighed once weekly at approximately 6 h postfeeding and the amount of milk replacer offered was adjusted accordingly. At approximately wk 7, a 7-d preweaning period was initiated in which the amount of milk replacer offered was reduced to 50% of the preweaning amount, and then after 7 d, the milk replacer was removed completely. A minimum BW of 70 kg was required for weaning. A textured starter was offered starting at approximately 3 wk into the study (Table 1). Calves had continual access to water throughout the entire milk-fed phase.

For both treatments and at each stage of growth, postweaning diets were formulated to be energy limited and CP excess. The CP content of the calf starter was based on unpublished growth data from our laboratory suggesting that the postweaning ADG could be more easily maintained at a higher CP level. Consistent with this, Gabler and Heinrichs (2003) fed heifers greater than 150 kg of BW with diets as high as 76.5 g of CP/

Table 2. Diet DM and nutrient composition of the TMR fed to postweaned heifers

Item	TMR ¹		
	1	2	3
Ingredient	(% of DM)		
Alfalfa hay	10.3	—	—
Alfalfa silage	15.1	31.8	31.5
Corn silage	13.8	31.8	31.5
SBM ²	—	—	4.0
Nonenzymatically browned SBM	10.3	18.0	7.9
Starter grain mix ³	50.3	—	—
High-moisture corn	—	15.9	22.6
Monensin mix ⁴	—	1.6	1.7
Vitamin E ⁵	0.12	0.10	—
Mineral mix ⁶	0.12	0.9	1.0
Nutrient			
CP	24.3	19.0	17.2
ADF	18.1	21.1	20.2
NDF	35.5	34.0	31.4
NSC ⁷	34.3	35.9	40.0
Fat	1.91	3.93	3.45

¹Total mixed ration 1 was offered to all heifers from 10 to 13 wk of age; TMR 2 was offered to all heifers from 13 wk of age until they reached 200 kg of BW; TMR 3 was offered to all heifers from 200 to 350 kg of BW.

²Soybean meal.

³Textured calf starter (Agway Inc., Syracuse, NY).

⁴Provides 1,760 mg of monensin (Elanco Animal Health, Greenfield, IN) per kg of DM.

⁵Supplement contained 44 IU/g of vitamin E.

⁶Supplement contained 58.6% cornmeal, 30.5% NaCl, 4.7% calcium sulfate, 1.9% calcium carbonate, 1.3% manganese sulfate, 1.1% zinc sulfate, 0.55% ferrous sulfate, 0.53% vitamin E (500 IU/kg), 0.33% copper sulfate, 0.21% magnesium oxide, 0.20% selenium (1.0%), 0.034% vitamin A, 0.022% vitamin D, 0.004% cobalt carbonate, and 0.004% iodine.

⁷Nonstructural carbohydrates, NSC = 100 - (ash + CP + NDF + ether extract).

Mcal of ME. Animals in the current experiment were started at a lighter BW and were therefore assumed to have an equivalent or higher protein requirement to ensure that protein was not limiting.

Throughout the entire study, postweaned heifers were weighed weekly and the amount of feed offered was adjusted to achieve the targeted rate of BW gain using the Cornell Net Carbohydrate and Protein System (Fox et al., 2004). Two sets of scales were used to weigh the heifers and were specific to the housing prior to and after weaning. All weigh scales at the research farm were calibrated monthly (Fairbanks Scale, Buffalo, NY). Postweaning BW were measured on the same day every week, between 0700 and 1000 h at the end of the feeding cycle. Fresh feed was delivered between 1000 and 1200 h once daily. For 2-wk following weaning, all heifers were offered only the textured starter (Table 1). Total mixed ration 1 (Table 2) was offered from 10 to 13 wk of age, and TMR 2 (Table 2) was offered from 13 wk until 200 kg of BW. Total mixed

ration 3 (Table 2) was offered from 200 to 350 kg of BW. From the initiation of treatment to 150 kg of BW, heifers were housed and fed in individual pens within a greenhouse structure. After 150 kg of BW, heifers were group housed and individually fed via the Calan gate system (American Calan, Northwood, NH).

Once heifers reached 225 kg of BW, blood was collected twice weekly via jugular venipuncture and the plasma progesterone concentration determined (Coat-A-Count progesterone radio immunoassay; Diagnostic Products Corp., Los Angeles, CA). Progesterone concentrations above 1 ng/mL were interpreted as the heifer possessing a functional corpus luteum and therefore pubertal.

Six heifers were harvested at 46 kg of BW to determine mammary development prior to the initiation of treatment. The remaining heifers (6 per treatment per harvest weight) were harvested at each of the following BW: 100, 150, 200, 250, 300, or 350 kg. Heifers were harvested at the Cornell University abattoir by stunning with a captive bolt followed by exsanguination. Pubertal heifers were harvested in the luteal phase of their reproductive cycle to minimize variation in PAR DNA content associated with estrus (Sinha and Tucker, 1969).

At harvest, the udder was removed, weighed, skinned, and separated into right and left halves at the medial suspensory ligament. The skin and teats from the whole udder were weighed together and the skinned right half was weighed separately. The weight of the skinned left half was determined by the difference.

The skinned right half was frozen on dry ice and stored at -20°C until further processing. At a later date, the right half was partially thawed at 4°C and cut into 5-mm-thick slices using a meat slicer. From these slices, the PAR, MFP, and supramammary lymph node were quantitatively dissected by color, collected, and weighed. The total PAR and MFP from each heifer were separately ground and subsampled for proximate analysis and determination of DNA content. The supramammary lymph node was weighed and discarded. Ground PAR and MFP subsamples were frozen in liquid nitrogen and further ground to a fine powder in a commercial blender.

The DNA content of the PAR and MFP was determined using the fluorometric bisbenzimidazole technique (Labarca and Paigen, 1980). Briefly, 0.25 mg of tissue was weighed and homogenized in a high-salt buffer (0.05 M Na_2PO_4 , 2.0 M NaCl, 2.0 M $\text{Na}_2\text{EDTA}\cdot 2\text{H}_2\text{O}$; Polytron PT3000, Brinkmann Instruments, Westbury, NY). Ten microliters of this homogenate was added to 1.89 mL of PBS (pH 7.4) to which 100 μL of Hoechst 33258 (1:50 dilution in PBS) was added. The DNA was quantified against a calf thymus DNA stan-

Table 3. Pre- and postweaning BW, age, and growth data for heifers fed 2 levels of nutrient intake from early life

Item	Treatment ¹		SEM
	E	R	
Initial BW, kg	43.9	44.4	0.7
Initial age, d	10.3	9.4	0.7
Weaning weight, kg	85.9 ^a	75.7 ^b	0.7
Length of milk-fed phase, d	43.3 ^a	49.7 ^b	1.2
Preweaning gain, kg/d	0.96 ^a	0.64 ^b	0.01
Lifetime gain, kg/d	0.93 ^a	0.66 ^b	0.01

^{a,b}Within rows, means with unlike superscripts are different ($P < 0.05$).

¹Treatment E = elevated nutrient intake; treatment R = restricted nutrient intake.

dard curve using a fluorometer with an excitation wavelength of 360 nm and an emission wavelength of 460 nm.

A subsample of powdered tissue was freeze-dried and used for additional analysis of lipid, CP, and hydroxyproline contents. The lipid content was determined by ether extract (AOAC, 1981). The nitrogen content was analyzed by a Kjeldahl procedure (AOAC, 1990) modified to include the use of boric acid and steam distillation (Pierce and Haenisch, 1940). The CP content was calculated as $\text{N} \times 6.25$. The hydroxyproline content of PAR was determined by HPLC following acid hydrolysis (Gehrke et al., 1985) and hexane extraction of lipids (Keene, 1996). Amino adipic acid served as the internal standard.

Statistical Analysis

Data were analyzed by the mixed-model procedure of SAS (SAS Institute, 2001). Sums of squares were partitioned to treatment (E or R), harvest weight, and treatment $\times$ harvest weight interaction. To facilitate data interpretation, the PDIF option of SAS was used to calculate means of main effects (treatment and harvest weight) as well as means of their interaction and their significance. Calf was the experimental unit. For each heifer, growth rate was determined by regressing weekly weights on time and taking the overall slope as the treatment ADG. Age and BW at puberty and number of ovulations were analyzed using the GLM procedure, with sums of squares partitioned to treatment. In all cases, when comparing means, an overall level of statistical significance was established at $P < 0.05$.

RESULTS

Body Growth

Preweaning and lifetime growth data are given in Table 3. Age and BW at initiation of treatment were

Table 4. Age, BW, and number of ovulations of heifers achieving puberty prior to harvest

Item	Treatment ¹				<i>F</i> -test <i>P</i> -value
	E		R		
	Mean	SEM	Mean	SEM	
Number reaching puberty	10	—	7	—	—
Age at puberty, d	245 ^a	7.6	353 ^b	9.1	<0.01
Weight at puberty, kg	270	6.6	289	10.4	0.09
Number of ovulations ²	3.4	0.73	4.6	0.87	0.32

^{a,b}Means with unlike superscripts are different ($P < 0.05$).

¹Treatment E = elevated nutrient intake; treatment R = restricted nutrient intake.

²Number of progesterone peaks >1.0 pg/mL between puberty and harvest.

not different between treatments. Daily BW gain during the milk-fed phase averaged 960 and 640 g/d for E- and R-heifers ($P < 0.01$). R-heifers were older at weaning than E-heifers because of the minimum weaning BW of 70 kg ($P < 0.05$). Lifetime ADG followed the same trend as preweaning ADG, with E-heifers gaining 930 g and R-heifers gaining 640 g ($P < 0.01$).

A total of 17 heifers reached puberty before they were harvested, 10 E-heifers and 7 R-heifers. Age at puberty was influenced by level of nutrient intake ($P < 0.01$); however, despite this difference in age, BW at puberty averaged 280 kg and tended to be affected only by treatment ($P = 0.09$, Table 4). The total number of progesterone peaks between puberty and harvest was unaffected by the plane of nutrition and averaged 3.4 and 4.6 per heifer for E- and R-heifers, respectively.

Body weight and age at harvest are presented in Table 5. As intended, treatment had no effect on BW at harvest; however, age at harvest was markedly affected by treatment, with E-heifers significantly younger than R-heifers at each of the 6 harvest BW.

Growth and Composition of the Mammary PAR

At 46 kg of BW, the epithelial component of the udder consisted only of a threadlike mass that extended dorsally above each teat. This structure was not easily excised, so it was left with the MFP and assumed to have a negligible impact on MFP weight and DNA content.

Total udder-half weight (skinned right half of the udder including both PAR and MFP) was increased in heifers on treatment E and with increased BW ($P < 0.01$; Table 5). Both PAR weight and DNA content were lower in E- than in R-heifers at all BW ($P < 0.01$). Likewise, E-heifers had significantly less PAR and PAR DNA per kg of BW than did R-heifers ($P < 0.01$).

As a result of the study design (with timing of harvest based on BW), E-heifers were younger at harvest than R-heifers ($P < 0.01$). To evaluate the effect this age

difference had on mammary development, age at harvest was used as a covariate in the statistical analysis of PAR DNA. This analysis demonstrated that age at harvest was a significant covariate term in this model ($P < 0.01$), and when used as a covariate, it accounted for nearly all variation in PAR DNA and reduced the treatment and BW effects to nonsignificant levels ($P = 0.75$ and 0.27 , respectively; Table 5). Use of age at harvest as a covariate term in the analysis of PAR DNA per kg of BW yielded similar results (data not shown).

Treatment did not influence the lipid, CP, or hydroxyproline composition of the mammary PAR (Figure 1 and Table 5). Significant developmental changes in PAR composition were observed, as the percentage of lipids and hydroxyproline increased linearly with BW and the percentage of protein decreased linearly with BW (Figure 1 and Table 5). The total amount of CP and lipids within the PAR followed similar trends as the PAR mass (Table 5). R-heifers retained more total lipids and protein within their mammary PAR than E-heifers ($P < 0.01$). Additionally, the total amount of each of these components increased as BW increased ($P < 0.01$).

Growth and Composition of the MFP

As previously discussed, the udder at 46 kg of BW consisted almost exclusively of MFP and the right-half MFP averaged 38.1 g (data not shown). E-heifers had increased MFP weight and MFP per kg of BW ($P < 0.01$). Increases in MFP weight were observed for all heifers as they increased in BW (Table 6). The interaction of the main effects was also significant for both total MFP weight and MFP per kg of BW ($P < 0.01$ and 0.04 , respectively), an observation we attribute to the reduced intake associated with training the heifers to the Calan gates. Level of nutrient intake and BW had a similar effect on MFP DNA and MFP DNA per kg of BW ($P < 0.05$), although this increase was not as pronounced as the increase in MFP weight, as E-heifers

Table 5. Effect of prepubertal nutrient intake on harvest weight, age at harvest, and mammary parenchyma (PAR) development among 6 harvest weights in heifers raised on an elevated (E) or restricted (R) plane of nutrition¹

Item	Harvest weight (kg) and treatment ²												<i>F</i> -test <i>P</i> -value			
	100		150		200		250		300		350					
	E	R	E	R	E	R	E	R	E	R	E	R	SEM	TRT ³	BW ⁴	Int. ⁵
BW, kg	102.7	104.8	152.0	151.8	205.0	204.7	255.0	255.8	303.8	299.8	359.7	361.2	2.0	1.00	<0.01	<0.71
Age at harvest, d	81.3 ^a	113.2 ^b	133.7 ^a	187.8 ^b	182.8 ^a	264.5 ^b	231.2 ^a	316.8 ^b	280.0 ^a	378.0 ^b	338.2 ^b	466.7 ^b	7.2	<0.01	<0.01	<0.01
Hemigland weight, g	140.7	124.5	284.9	280.2	563.7	667.3	1,029.2	792.4	1,321	1,147	1,838 ^a	1,451 ^b	91.9	0.02	<0.01	0.06
PAR, g	17.5	30.3	62.7	122.2	158.0	275.1	315.3	393.6	367.3	535.7	551.7	715.1	41.3	<0.01	<0.01	0.27
PAR, g/kg of BW	16.9	28.8	41.3	80.5	77.1	133.9	123.3	153.8	120.7	178.1	152.7	197.8	0.14	<0.01	<0.01	0.46
DNA, mg	33.5	63.0	129.5	276.5	354.3 ^a	708.7 ^b	623.5 ^a	985 ^b	710.7 ^a	1,121 ^b	848.9 ^a	1,351 ^b	97.9	<0.01	<0.01	0.10
Covariate DNA, ⁶ mg	751.7	644.6	623.2	537.8	637.2	641.2	698.9	693.7	576.7	566.6	465.4	416.9	350.3	0.75	0.27	0.99
DNA, mg/kg of BW	0.33	0.60	0.85	1.82	1.73	3.44	2.44	3.85	2.34	3.72	2.35	3.74	0.35	<0.01	<0.01	0.32
Ether extract, g	2.3	4.6	12.5	23.7	42.8	90.2	125.2	161.1	158.7	234.4	228.7	340.2	19.4	<0.01	<0.01	0.34
Protein, g	1.8	3.0	6.1	12.7	14.8	25.3	26.9	35.5	34.6	47.3	39.4	59.6	4.9	<0.01	<0.01	0.42
OH-Pro, ⁷ mg/g of tissue	2.81	2.84	3.11	3.45	3.15	2.52	2.02	3.07	3.19	3.80	3.21	4.92	0.62	0.12	0.10	0.42

^{a,b}Means with unlike superscripts within a common harvest weight are different ($P < 0.05$).

¹All values represent the right hemigland.

²Treatment E = elevated nutrient intake; treatment R = restricted nutrient intake.

³Treatment, elevated vs. restricted plane of nutrition.

⁴Harvest weight.

⁵Interaction of main effects. Interaction means were separated using the PDIF options of SAS (SAS Institute, 2001) for comparisons within harvest weight groups when the *F*-test indicated a potential interaction ($P < 0.20$).

⁶Total DNA analyzed with age at harvest as a covariate. Significance of age at harvest as a covariate term, $P < 0.01$.

⁷Hydroxyproline on an as-is basis.

Figure 1. Means (SEM) for ether extract (upper panel) and protein (lower panel) composition of the mammary parenchyma (on a wet basis) of heifers across 6 harvest weights raised on an elevated (solid bars) or restricted (open bars) plane of nutrition. Significance of main effects and their interaction for ether extract composition: treatment, $P = 0.14$; harvest weight, $P < 0.01$; interaction, $P = 0.47$. Significance of main effects and their interaction for protein composition: treatment, $P = 0.40$; harvest weight, $P < 0.01$; interaction, $P = 0.58$.

had significantly less DNA per unit of MFP than did R-heifers (data not shown).

Differences in age at harvest between the 2 treatments did not describe any variation in total MFP DNA or MFP DNA per kilogram of BW because age at harvest was not a significant covariate term in the analysis of these 2 variables ($P = 0.48$ and 0.96 , respectively; data not presented).

Both treatment and BW significantly influenced the fractions of CP and lipids in the MFP ($P < 0.01$; Figure 2). In each case, the interaction between harvest weight and treatment was also significant ($P < 0.05$). Increased nutrient intake for heifers on treatment E reduced the percentage of CP in the MFP, whereas it increased the percentage of lipids ($P < 0.01$). This effect of treatment on protein and lipid partitioning was most pronounced at the 100- and 150-kg harvest weights.

E-heifers retained more total CP and lipids in their MFP than did R-heifers, and in all cases, the amounts of both components increased as BW increased, with

Table 6. Effect of prepubertal nutrient intake on mammary fat pad (MFP) development across 6 harvest weights in heifers raised on an elevated (E) or restricted (R) plane of nutrition¹

Harvest weight (kg) and treatment ²																					
100			150			200			250			300			350			F-test P-value			
Item	E	R	E	R	E	R	E	R	E	R	E	R	E	R	SEM	TRT ³	BW ⁴	Int. ⁵			
MFPP, g	116.9	88.9	214.0	147.2	381.7	370.4	691.7 ^a	379.3 ^b	920.0 ^a	580.2 ^b	1126 ^a	710.0 ^b	83.0	<0.01	<0.01	<0.01	<0.01				
MFPP, g/kg of BW	1.14	0.84	1.41	0.97	1.86	1.81	2.7 ^a	1.48 ^b	3.04 ^a	1.94 ^b	3.49 ^a	1.96 ^b	0.29	<0.01	<0.01	<0.01	0.04				
DNA, mg	73.0	65.6	116.6	96.8	149.8	160.8	236.6	192.0	298.9	215.9	539.0 ^a	323.7 ^b	51.6	0.03	<0.01	0.19					
DNA, mg/kg of BW	0.71	0.62	0.77	0.64	0.73	0.78	0.93	0.75	0.98	0.72	1.50	0.89	0.16	0.02	<0.01	0.31					
Ether extract, g	64.7	38.4	131.6	74.0	254.6	252.9	496.4 ^a	255.6 ^b	688.7 ^a	388.1 ^b	989.1 ^a	515.9 ^b	70.4	<0.01	<0.01	<0.01	<0.01				
Protein, g	10.5	9.9	16.1	14.1	25.6	22.8	37.9 ^a	24.6 ^b	49.1 ^a	36.8 ^b	53.5 ^a	42.0 ^b	3.2	<0.01	<0.01	<0.01	0.09				

^{a,b}Means with uncommon superscripts within a common harvest weight are different at $P < 0.05$.

¹All values represent the right hemigland.

²Treatment E = elevated nutrient intake; treatment R = restricted nutrient intake.

³Treatment, elevated vs. restricted plane of nutrition.

⁴Harvest weight.

⁵Interaction of main effects. Interaction means were separated using the PDIF options of SAS (SAS Institute, 2001) for comparisons within harvest weight groups when the F -test indicated a potential interaction ($P < 0.20$).

Figure 2. Means (SEM) for ether extract (upper panel) and protein (lower panel) composition of the mammary fat pad (on a wet basis) of heifers across 6 slaughter weights raised on an elevated (solid bars) or restricted (open bars) plane of nutrition. Significance of main effects and their interaction for ether extract composition: treatment, $P < 0.01$; harvest weight, $P < 0.01$; interaction, $P = 0.02$. Significance of main effects and their interaction for protein composition: treatment, $P < 0.01$; harvest weight, $P < 0.01$; interaction, $P = 0.04$. Within harvest weights, an asterisk (*) indicates a plane of nutrition effect ($P < 0.05$) at the specific harvest weight.

differences being most pronounced at heavier BW ($P < 0.01$; Table 6).

DISCUSSION

In this study, the rate of BW gain was increased by increasing nutrient intake. For all variables of interest, the effects of treatment were evaluated at a common BW but at different chronological ages. This difference in age is an outcome of the experimental design, which permitted comparisons to be made at a common physiological age. It is accepted that onset of puberty (i.e., physiological age) is a function of BW, body composition, or both rather than chronological age. This was demonstrated by the observation that elevated nutrient intake reduced the age at puberty but had little influence on

BW at puberty (Capuco et al., 1995; Niezen et al., 1996; Radcliff et al., 1997). When considering the subset of heifers reaching puberty in the current study, E-heifers reached this developmental stage at a chronological age that was 108 d younger than R-heifers. Despite this marked difference in age at puberty, BW at puberty was similar between the 2 treatments. Therefore, although E- and R-heifers had different chronological ages at a common BW, it can be assumed that they were of similar physiological and developmental ages.

It was our intention to ensure that diets in the current study were not protein limiting. Although this resulted in feeding higher levels of dietary protein than conventionally recommended, it can be assumed to have a limited influence on the outcome of the study because the protein source or level of dietary protein rarely influences mammary development (Mantysaari et al., 1995; Dobos et al., 2000; Whitlock et al., 2002; Capuco et al., 2004).

This data set is the first to characterize the effects of level of nutrient intake on development of the prepubertal bovine PAR and MFP at multiple points from birth through puberty. Although it is apparent that MFP is directly influenced by level of nutrient intake, this appears not to be the case for PAR, as discussed below.

Parenchyma weight and DNA content were significantly reduced in E-heifers when assessed at a common BW. This finding is consistent with most other data in the literature when the effect of nutrient intake is assessed at a common BW (Sejrsen et al., 1982; Petitclerc et al., 1984; Capuco et al., 1995). However, this study was the first to assess variation in PAR DNA attributable to differences in age at harvest, which are inherently associated with experiments of this design. Specifically, these data demonstrated that use of age at harvest as a covariate eliminated the effect of nutrition on PAR DNA content, suggesting that at a common BW, most of the variation in PAR DNA is a reflection of differences in age at harvest.

Similar covariate analysis of MFP DNA did not hold true, because age at harvest was not a significant covariate term in the statistical model. This suggests that, at a common BW, age is not a major determinant of MFP development. Therefore, although increased nutrient intake directly influenced MFP growth, its influence on PAR development was indirect and time dependent. Elevated nutrient intake simply reduced the length of time the gland had to grow between birth and the BW chosen to assess mammary development. This effect of time, or chronological age, on organ growth was also observed in testicular development in lambs. Greenwood et al. (2004) observed that when compared at a common BW, lambs raised on a high plane of nutrition

had smaller testes than cohorts raised on a restricted level of nutrient intake. Such observations suggest that although some tissues are responsive to increased nutrient intake (e.g., adipose tissue increases in size), this appears not to be the case for reproductive organs, including the testis and PAR.

As previously reported, increased nutrient intake resulted in larger MFP (Sejrsen et al., 1982; Capuco et al., 1995; Radcliff et al., 1997). Although MFP weight was increased by an elevated level of nutrient intake by 100 kg of BW in the current study, differences in MFP DNA at similar BW were not apparent until 350 kg of BW. This suggests diet-induced hypertrophy of MFP adipocytes. This hypertrophy is presumably brought about by increased partitioning of nutrients to the MFP, resulting in lipid engorgement of adipocytes. This conclusion is supported by observed increases in lipid composition and decreases in CP composition of the MFP. An effect of treatment on the lipid and CP composition of the PAR was not observed, suggesting that this tissue is refractory to the homeorhetic signals that coordinate increased lipid deposition in the MFP, and is consistent with observations of Sejrsen et al. (1982). Further support for this conclusion is the observation that the PAR histological composition of E-heifers was similar to that of R-heifers. Specifically, Daniels et al. (2005) detected no effect of elevated nutrient intake on the area occupied by inter- and intralobular stroma (including adipocytes), epithelium, and lumen or in the number of luminal and epithelial structures in the PAR in E- compared with R-heifers.

CONCLUSIONS

Data presented herein suggest that the MFP is directly influenced by elevated nutrient intake; however, the same is not true for PAR. Although MFP adipocytes respond to signals coordinating nutrient partitioning by undergoing hypertrophy and ultimately hyperplasia, those in the PAR appear refractory, because the effects of treatment had no influence on the lipid content of the PAR. Additionally, development of epithelial structures or their ability to displace adipocytes during invasion into the MFP was unaffected by treatment. Last, the finding that most of the variation in PAR DNA content was accounted for by differences in age, not the level of nutrient intake, supports these conclusions and suggests that age at harvest was the primary determinant of prepubertal mammary development in the heifer.

ACKNOWLEDGMENTS

The authors wish to thank Joe McFadden, Matt Miller, Denny Shaw, Bruce Berggren-Thomas, Jenny

Kelsey-Mills, and Erin Peterson for their help with animal care and tissue collection. We would also like to thank Jeff Tikofsky, Agway Feed and Nutrition (Syracuse, NY), and Mike Fowler, Land O'Lakes Animal Milk Products Company (St. Paul, MN), for their support of this experiment. This work was also partially funded by the Cornell University Agricultural Experiment Station and USDA-ARS. This research is a component of NC-1119: Management Systems to Improve the Economic and Environmental Sustainability of Dairy Enterprises.

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