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Effects of compensatory growth on the expression of milk protein gene and biochemical changes of the mammary gland in Holstein cows

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Twenty-four Holstein heifers were randomly assigned to either a control or test group to investigate effects of an incremental growth pattern on the development and differentiation of the mammary gland, expression of milk protein gene, and improvement of productivity. The test group was fed the restriction diet (RD; 20% below that of the control group for all nutrients) for 3 months, followed by the compensatory diet (CD; 25% above that of the control group for all nutrients) for 2 months, then followed by 4-month RD, 2-month CD, 5-month RD, and finally 2-month CD. The heifers on the dietary test regimen gained more body weight and consumed less diet, resulting in improved growth rate efficiency, compared with heifers in the control group (8.3% vs. 7.8%). The amount of retained protein and secreted protein in mammary acinar cell culture was higher for heifers in the test group than for those in the control group (midpregnancy, 2.5 and 1.9 times; late pregnancy, 1.3 and 1.1 times, respectively). RNA dot blot hybridization analysis revealed that accumulation of α_{S1}-casein and β-casein mRNA in mammary gland tissue from heifers in the test group was higher (α_{S1}-casein, 1.37 times; β-casein, 1.74 times) than those from heifers in the control group. Total activity of RNA polymerases and activity of RNA polymerase III in mammary tissue were higher in heifers in the test group than in those in the control group. Heifers in the test group had lower cyclic adenosine monophosphate concentrations than did heifers in the control group during all periods. The incremental growth pattern decreased concentrations of 5′ methyldeoxycytidine (5 mdc) in the mammary gland. The incremental growth pattern offered not only a simple, practical, and cost-effective method for raising dairy heifers but also may have contributed to an increase in the differentiation and functional activity of the mammary gland. (J. Nutr. Biochem. 9:380–387, 1998) © *Elsevier Science Inc. 1998*

Keywords: compensatory growth; milk protein gene; cAMP; heifers

Introduction

Compensatory growth occurs when previously marginally fed or underfed animals are fed a diet with higher nutritional value. Compensatory growth induced by an incremental nutrient regimen significantly enhances the efficiency of body growth and subsequent lactational performance.[1] Several studies[2–4] have emphasized the importance of nutrition in mammary development and subsequent lactation. During the compensatory growth period, animals exhibited greater daily body weight gain, increased appetite and nutrient utilization, reduced maintenance requirement (by depressing the basic metabolic rate), enhanced feed intake capacity, activated endocrine status, changes in the composition of

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Received January 8, 1997; accepted March 24, 1998.

655 Avenue of the Americas, New York, NY 10010
0955-2863/98/$19.00
PII S0955-2863(98)00027-8

Table 1 Ingredient and chemical composition of experimental diets

Variable	Maintenance phase		Compensatory phase	
	Control	Restriction	Control	Compensatory
Ingredient (kg/d of DM)				
Period 1[1]				
Corn silage	1.76	1.78	2.60	2.52
Hay[4]	.69	.76	.88	.74
Concentrate	2.88	1.48	3.05	4.58
CP of diet	.68	.47	.79	1.01
Period 2[2]				
Corn silage	3.00	3.00	5.22	5.22
Hay	1.78	1.78	2.67	2.67
Concentrate	3.63	2.31	1.34	3.13
CP of diet	1.02	.80	.92	1.21
Period 3[3]				
Corn silage	5.22	5.22	5.19	5.16
Hay	2.67	2.67	3.52	3.52
Concentrate	2.06	1.16	1.75	3.06
CP of diet	1.03	.89	1.08	1.30
		Silage	Hay	Concentration
Chemical analysis of diet				
DM (%)		29.88	87.92	87.30
CP (% of DM)		7.65	11.23	16.20
Crude fat (% of DM)		2.94	1.80	2.50
Crude fiber (% of DM)		23.48	31.87	4.47

[1]Heifers were fed the restriction diet (feed intake adjusted to 20% below requirements) for 3 months followed by the compensatory diet (feed intake adjusted to 25% above requirement) for 2 months.
[2]Heifers were fed the restriction diet (feed intake adjusted to 20% below requirements) for 4 months followed by the compensatory diet (feed intake adjusted to 25% above requirement) for 2 months.
[3]Heifers were fed the restriction diet (feed intake adjusted to 20% below requirements) for 5 months followed by the compensatory diet (feed intake adjusted to 25% above requirement) for 2 months.
[4]Hay was composed of 30% alfalfa and 70% orchardgrass.
CP, crude protein; DM, dry matter.

body tissue gain, and improved overall efficiency when compared with conventionally fed animals during a similar period.[5–7]

Compensatory growth in female rats resulted in increased expression of the milk protein genes because of increased mRNA transcripts, and increased protein synthesis and secretion of milk protein.[8] Baik[9] reported that compensatory growth increased milk production by 10%. Using cDNA probes for specific milk protein genes, progress has been made in the area of gene expression, gene modulation, and release of differentiated products of mammary epithelial cells. The regulation of the expression of milk protein gene has recently been analyzed at the molecular level by probing casein, whey acidic protein, and α-lactalbumin mRNA with respective cDNA.[10] Moreover, in vitro culture systems that allow extended growth of mammalian cells are powerful tools for the study of cell proliferation and gene expression. Park et al.[8] found that compensatory growth induced by an incremental nutrient regimen modulated expression of the milk protein gene at the cellular and molecular level, including milk protein mRNA, protein synthesis, and secretion. However, the role of compensatory growth in bovine mammary cell growth and differentiation is not well understood.

Therefore, the objective of this research was to determine effects of compensatory growth on growth performance, expression of the milk protein gene, and functional development of the mammary gland in Holstein heifers.

Materials and methods

Heifers

Twenty-four Holstein heifers [6 months-old and 185.6 kg of body weight (BW)] were equally and randomly assigned to either a control or test group. The heifers were individually housed and managed in 2.4 m by 2.4 m pens with concrete slatted floors. The control group was fed a control diet to meet National Research Council[11] nutrient recommendations for heifers. The experimental period was approximately 18 months and was followed by a 2-month standardization period during which all heifers received the control diet. The test group was subjected to a stair-step growth pattern and was fed according to a schedule of 3, 2, 4, 2, 5, and 2 months; periods of 3, 4, and 5 months of maintenance were alternated with 2 months of compensatory growth during which feed intake was alternately 20% below or 25% above controls, respectively. During maintenance periods, nutrient and feed intake were controlled by the amount of concentrate fed. Ingredients and chemical composition of treatment diets are presented in *Table 1*. The concentrate diet consisted of 42.3% corn, 3.0% wheat grain, 8.0% corn gluten, 16.0% wheat bran, 6.0% cottenseed meal, 8.6% soybean meal, 5.0% rapeseed meal, 3.0% palm kemmel meal, 4.7% molasses, and 3.43% minerals mix. The forage used was 66% corn silage, 10.2% alfalfa hay, and 23.8% orchardgrass hay. The amount of diet fed was weighed before the morning feeding, and feed intake was recorded once daily. Water was provided ad libitum, and dicalcium phosphate and trace-mineralized salt were available for free choice intake at all times. All heifers were bred during the compensatory period (14 to 16 months of age) to calve at 23 to 25 months of age. Heifers were weighed every month.

Mammary tissue samples

Pregnant heifers from each group at midpregnancy (2 animals/group) and late pregnancy (2 animals/group) were anesthetized with 1 mL of 2% Rompun (Bayer, Seoul, Korea). After local anesthesia with lidocaine, a 4-cm incision was made at the left rear mammary quarter, and a tissue sample of approximately 10 g was removed from as deep as possible by sharp dissection. A part of mammary tissue was bathed in sterile balanced salt solution containing antibiotics (penicillin, 10^4 IU; amphotericin-B, 250 μg; streptomycin, 10^4 IU/L) and calf serum, and was transported to the laboratory on ice for alveolar cell culture. The rest of mammary tissue was frozen immediately in liquid nitrogen. Following surgery, heifers were given 8×10^6 IU of penicillin by intramuscular injection twice a day for 3 days.

Preparation of mammary tissue for SEM

Samples for observation using scanning electronmicroscopy (SEM) were prepared by modifying procedure of Caruolo.[12] After fixation at 4°C for 2 hours in PBS [0.2 *M* phosphate buffer (pH 7.0) and 0.2 *M* sucrose containing 5% glutaraldehyde], mammary tissue was washed with 0.1 *M* PBS at 4°C for 20 minutes. The sample was dehydrated with ethanol and isoamylacetate and dried with liquid carbon dioxide. Dried samples were coated with gold for 2 minutes then observed using a scanning electron microscope (Hitachi S-570, Hitachi Ltd., Tokyo, Japan).

Protein synthesis and secretion activity in mammary acinar culture

Cell dispersion was performed according to the method of Raber and D'Ambrosio.[13] Mammary tissue was minced with sterile scalpels and scissors to less than 5 mm^3, and tissues were stirred gently at 37°C for 3 hours in a solution containing 400 U/mL of collagenase (Type I) and 400 U/mL of hyaluronidase. The cells were plated on plastic tissue culture dishes (approximately 10^7 cells/dish; 35 × 10 mm). The basic medium used was Eagle's minimum essential media (MEM) as modified by Smith et al.[14] Glucose and bovine serum were added to 1 × MEM to a final concentration of 0.2% (wt/vol) and 5% (vol/vol), respectively. The media was supplemented with antibiotics (penicillin, 10^4 IU; amphotericin-B, 250 μg; and streptomycin, 10^4 IU/L of media). The pH of the media was adjusted to 7.4 by addition of 7.5% sodium bicarbonate. The isotope used for labeling the protein to be synthesized in the cell culture was [^{3}H]-lysine (0.5 μCi/mL). At the termination of the 18-hour incubation, cells were collected, pooled, and centrifuged at 1000 × g at 4°C for 10 minutes. The amount of synthesized and secreted milk protein was measured according to Choi.[15] The equation used to calculate synthesized protein was as follows:

$$P = [C/E]/A$$

where P is the specific activity (dpm/mg of protein), C is the cpm of [^{3}H]-lysine incorporated in protein, E is the isotope counting efficiency, and A is the amount of protein (mg).

Specific activity of [^{3}H]lysine incorporated into protein was counted by a liquid scintillation counter.

cAMP assay. Concentration of cyclic adenosine monophosphate (cAMP) in the mammary gland tissue was measured by a radioimmunoassay kit (RIA kit; Amersham, Arlington, IL, USA). Frozen tissue samples were homogenized with cold 6% trichloroacetic acid and centrifuged at 2000 × g for 15 minutes. Supernatant was extracted four times with 5 volumes of diethyl ether saturated with water (95:1, vol/vol). The extract was dried and dissolved in assay buffer for immunoassay.

RNA extraction. Total cytoplasmic RNA of the mammary gland tissue was extracted by the acid guanidium thiocyanate-phenol-chloroform extraction method.[16] RNA dotting was performed according to the procedure of White and Bancroft.[17] The 6 μg of RNA were denatured and transferred to nylon membrane (Amersham) by 96-well filtration manifold. The membrane was baked at 80°C for 2 hours.

Hybridization. Bovine α_{S1}-casein and β-casein cDNA were donated by A.G. Mackinlay. The cDNA were cloned in *Pst* I site of pBR322. The plasmids were isolated from the culture by CsCl ultracentrifugation. Probes were labeled according to the procedure of Digoxigenin-11-ddUTP DNA labeling and detection kit (Boehringer Mannheim Biochemica, Mannheim, Germany).

Specificities of α_{S1}-casein and β-casein probes used in these experiments were confirmed by Northern analysis hybridization, which was carried out at 65°C. The membrane was prehybridized with hybridization solution [5 × SSC, 0.02% BSA, 0.02% polyvinylpyrrolidine, 0.02% ficoll, 0.1% (wt/vol) N-laurylsarcosine, and 0.02% (wt/vol) SDS] and then hybridized with DNA probes for 12 hours. The membrane was washed twice with 2 × SSC (0.1% SDS) at room temperature (24°C) and then washed twice with 0.1 × SSC (0.1% SDS) at 68°C. Color reaction was developed with nitro blue tetrazolium and X-phosphate solution and terminated with TE (Tris-EDTA, pH 8.0). Specific activity of mRNA was measured by densitometry.

RNA polymerase activity

The activity of RNA polymerase was determined by the incorporation of [^{3}H]UTP into acini.[18] Mammary gland tissue was homogenized in buffer [(0.01 *M* HEPES, 10 μ*M* $CaCl_2$, and 1 *M* hexylen glycol (pH 7.0)] and centrifuged at 24,000 rpm for 1 hour (Beckman SW 41; Beckman Instruments, Somerset, NJ, USA) in 2.1 *M* sucrose solution [2.1 *M* sucrose, 0.05 *M* Tris-Cl, 0.01 m*M* DTT, and 1 m*M* $MgCl_2$ (pH 7.5)]. The precipitates were resuspended with 60 μL of reaction solution [50 m*M* Tris-Cl (pH 7.9), 50 m*M* $(NH_4)_2SO_4$, 0.183 m*M* $MnCl_2$, 2 μCi/assay [^{3}H]UTP, 0.6 m*M* GTP ATP CTP, and 10 m*M* UTP] with α-amanitin or actinomycin at 37°C for 10 minutes. After the solution was spotted on ED 81 filter and the cpm of [^{3}H]UTP on the filter was washed with 0.5 *M* Na_2HPO_4, distilled water, 95% ethanol, and ether. Dried filter was detected by a scintillation counter.

For determination of RNA polymerase I, II, and III activity, the following reaction conditions were included: (1) incubation without α-amanitin and actinomycin D, (2) incubation with 0.16 μg/mL of α-amanitin, (3) incubation with 320 μg/mL of α-amanitin, and (4) incubation with 10 μg/mL of actinomycin D. Activity of RNA polymerase I was calculated by the difference between conditions 3 and 4; activity of RNA polymerase II was calculated by the difference between conditions 1 and 2; and activity of RNA polymerase III was calculated by the difference between conditions 2 and 3.[18]

DNA methylation

Genomic DNA was extracted from mammary gland tissue according to the modified method of Sambrook et al.[19] Mammary gland tissue was homogenized in extraction buffer [10 m*M* Tris-Cl (pH 8.0), 0.1 *M* EDTA, 20 μg/mL of RNase, and 0.5% SDS] by using a homogenizer and then incubated at 37°C for 1 hour. After the addition of proteinase K to a final concentration of 100 μg/mL, the sample was incubated at 50°C for 3 hours and extracted with phenol, phenol-chloroform, and chloroform; DNA then was precipitated with ethanol. Precipitates were resuspended in TE buffer (pH 8.0).

Genomic DNA was digested with nuclease P1 and alkaline

Table 2 Overall growth performance of test heifers over the entire experimental period

	Control group	Test group						SEM[1]	Probability[2]
		Restriction	Compensatory	Restriction	Compensatory	Restriction	Compensatory		
Duration (mo)	18	3	2	4	2	5	2		
BW (kg)									
Initial	188	183.1	233.4	327.4	362.6	430.2	477.9	2.1	0.012
Final	562	233.4	327.4	362.6	430.2	477.9	568.1	10.2	0.018
Gain	373.2	50.3	94.1	35.1	67.6	47.7	90.2	3.7	0.021
Daily BW gain, kg/d	0.66	0.50	1.54	0.28	1.12	0.31	1.48	0.10	0.019
DMI, kg/d	8.47	4.02	7.84	7.09	11.02	9.05	11.74	0.21	0.046
Growth efficiency (%)[3]	7.79	12.4	19.6	3.9	10.2	3.4	12.6	1.1	0.027

[1]Standard error of the mean (SEM), where $n = 10$.
[2]Significance level of *T*-test for equality of six growth phases.
[3]Growth efficiency = daily body weight (BW) gain/daily mass intake (DMI) × 100.

phosphatase to produce deoxynucleosides. The digests were injected onto a reversed phase high performance liquid chromatography (HPLC) column (DEAE-sephadex A25 column; Pharmacia LKB Biotech., Piscataway, NJ, USA) and eluted with KH_2PO_4 (pH 4.0) and 2.5% methanol at a flow rate of 2 mL/min. Nucleosides were detected by the absorbance at 254 nm. The peaks were identified by comparison of elution time with those of standards. The relative portions of the individual nucleosides were calculated from the area under the peaks of the absorbance trace and extinction coefficients of the nucleosides. The percentage of 5′ methyldeoxycytidine (5 mdC) was calculated according to the following formula:

$$5\text{ mdC }(\%) = 5\text{mdC}/(5\text{ mdC} + \text{deoxycytidine}) \times 100.$$

Data analysis

All data were analyzed by the general linear models procedure of SAS.[20]

Results and discussion

Growth performance

During the 18 months, the BW of Holstein heifers that were nourished under the rotating restriction and compensatory feeding system showed an incremental growth pattern (*Table 2*). The respective average daily gains (ADG) of heifers in the test group were 0.50, 1.54, 0.28, 1.12, 0.31, and 1.48 kg for the corresponding experimental periods. During the first step of the restriction and compensatory feeding system, ADG was 0.92 kg for the control group and 0.96 kg for the test group. During the second duration, ADG were 0.54 kg and 0.57 kg, and during the third duration, ADG were 0.65 kg and 0.66 kg for the control and test groups, respectively. During the entire experimental period, ADG of the test group (0.68 kg) was slightly higher than that of the control group (0.66 kg). The dry matter intake (DMI) was lower for the heifers in the test group (8.21 kg) than for those in the control group (8.47 kg). Heifers in the test group consumed 1.7 metric tons less than did the control heifers for 18 months. Previously, Wilson and Osbourn[7] demonstrated that calves fed for compensatory growth showed high growth performance and feed efficiency. Park et al.[1] reported that although Holstein heifers had reduced BW during the restriction period, showed increased growth performance, energy, and protein efficiency during the compensatory period. In this study, the increase in growth performance of animals may be due to the improved energy and protein efficiency, decreased maintenance requirement, and an activated endocrine status by compensatory feeding system, which has been suggested by previous studies.[3,9,15,21,22]

Mammary gland development

The observations by SEM (*Figure 1*) showed that the alveolar system was more developed in late pregnancy than in midpregnancy. Parenchymal tissue of the test group was more developed than that of the control group. Our study suggests that a stair-step dietary regimen contributes to better development of mammary parenchymal tissue. Heifers raised on high planes of nutrition during the allometric phase of mammary development exhibit less secretory tissue in the mammary gland than do those raised at a normal growth rate.[22–25] Johnson and Hart[26] hypothesized that a high plane of nutrition might decrease the rate of allometric growth of mammary parenchyma. Negative influence of excess energy intake on mammary development of heifers in prepuberty may be associated with a decrease in the growth hormone concentration.[23]

Protein synthesis and secretion activities in mammary acinar cell culture

Protein synthesis and secretion activities of both groups in mammary acini culture were increased more in late pregnancy than in midpregnancy (*Table 3*). In protein synthesis, the test group produced 2.5 and 1.9 times higher protein than did the control group in midpregnancy and late pregnancy, respectively, and protein secretion of the test group was secreted slightly higher (1.3 and 1.1 times) than that of the control group in midpregnancy and late pregnancy, respectively. A previous study[15] reported that the compensatory growth pattern promoted approximately a 13% increase in amino acid uptake in rats in the late lactating stage. In acinar cell culture, protein synthesis and secretion of rats fed for compensatory growth were 1.3 and 1.2 times higher than those of control rats during pregnancy.[21] Researchers[9,15] reported that animals fed on incremen-

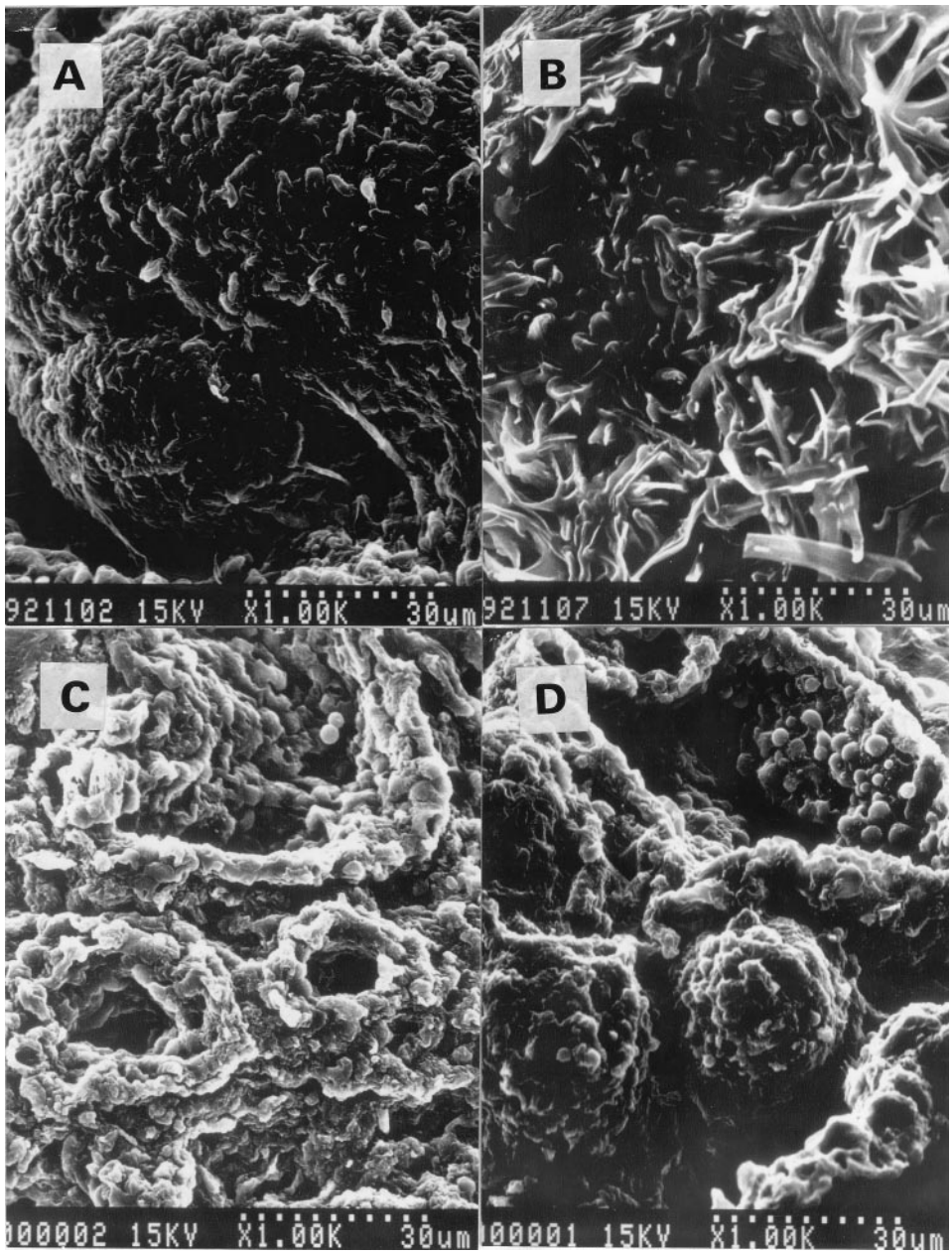

Figure 1 Scanning electron microscopy of mammary gland in 5-month and 8-month pregnancy. (A) Mammary gland of heifer in control group in midpregnancy; (B) mammary gland of heifer in test group in midpregnancy; (C) mammary gland of heifer in control group in late pregnancy; and (D) mammary gland of heifer in test group in late pregnancy.

Table 3 Protein synthesis and secretion in bovine mammary acini culture

Item	Midpregnancy		Late pregnancy		SEM[1]
	Control	Test	Control	Test	
Retained protein[2] (dpm/mg of protein)	11.54	29.30	86.80	164.13	13.02
Secreted protein[3] (dpm/mg of protein)	13.13	17.53	142.67	156.69	3.08

[1]Each value is the mean of eight determinations (four culture dishes per determination) made from mammary tissue of two pregnant heifers. SEM, standard error of the mean, where $n = 32$.
[2]Amount of retained protein was determined by the incorporation of [^{3}H]lysine into acini.
[3]Amount of secreted protein was determined by the incorporation of [^{3}H]lysine into trichloro acetic acid-insoluble material.

Table 4 Milk protein mRNA in bovine mammary gland

Specific activity[1]	Midpregnancy		Late pregnancy		SEM[2]
	Control	Test	Control	Test	
α_{S1}-casein	17.3	18.2	30.5	41.7	1.8
β-casein	15.6	16.5	18.7	32.6	2.4

[1]Specific activity = relative intensity/μg of RNA. Relative intensity was determined by scanning densitometry of individual dots.
[2]Standard error of the mean (SEM), where $n = 4$.

tal growth showed higher milk protein synthesis and secretion, and Park and Bissel[27] reported that milk protein synthesis was increased by insulin-stimulated amino acid uptake in mammary epithelial cell culture. In this study, the compensatory growth pattern also stimulated protein synthesis and secretion activities in mammary gland of Holstein heifers. This suggests that compensatory growth improves functional differentiation of mammary gland.

Expression of the milk protein gene

Both mRNA levels were increased in late pregnancy compared with heifers in midpregnancy (*Table 4*). In midpregnancy, both groups had similar levels of casein mRNA, but in late pregnancy α_{S1}-casein mRNA was 37% higher in the test group than in control group, and β-casein mRNA was 74% higher in the test group than in the control group. This result is consistent with previous reports[8,9,15] in which an incremental growth pattern has improved expression of the milk protein gene by increasing the level of mRNA. Previous reports[8,15] have indicated that cytoplasmic α_{S1}-, α_{S2}-, β-, and κ-casein mRNA levels were increased for animals fed for incremental growth. These higher levels of mRNA in the mammary gland of heifers in the test group might ensure a greater primary biological function of the gland. These increases in the relative abundance of mRNA by compensatory growth could have been attributable to either an increased transcription rate or a decreased rate of mRNA degradation in alveolar cells, or both. These results may imply that an incremental feeding regimen regulates the expression of the milk protein gene and other differentiated functions in acinar culture as well as in vivo.

RNA polymerase activity

RNA polymerase was activated more in late pregnancy than in midpregnancy (*Table 5*). The total activity of RNA polymerases was higher in the test group than in the control group at midpregnancy and late pregnancy. The nucleus of eucaryotes contains three types of RNA polymerases differing in template specificity and susceptibility to indicators. RNA polymerase I transcribes the tandem array of genes for 18S, 5.8S, and 28S ribosomal RNA.[28] The other ribosomal RNA (5S rRNA) and all of the transfer RNA molecules are synthesized by RNA polymerase III.[29] mRNA are synthesized by RNA polymerase II.[30] The present study revealed that RNA polymerase I and II were activated more in late pregnancy than in midpregnancy. Activity of RNA polymerase I in the test group was higher than that in the control group in midpregnancy, whereas that in the test group was slightly lower than the control group in late pregnancy. The test group showed RNA polymerase II activity lower than in the control group. The activity of RNA polymerase III was higher in the test group than in the control group at midpregnancy and late pregnancy.

Induction of milk protein gene expression is controlled by hormonal and enzymatic stimulations of transcriptional events, RNA processing and nucleocytoplasmic transport efficiency, and rate of mRNA degradation.[31] RNA polymerase is the key enzyme implicated in the transcription of DNA to RNA.[30] In this study, increase in total activity of RNA polymerase may imply that compensatory growth regimen stimulates transcription of gene (e.g., milk protein gene) expression in mammary gland.

cAMP Contents in mammary gland

Test group showed lower cAMP levels compared with the control group (*Figure 2*). cAMP in guinea pig,[32] rat,[33] and mouse mammary glands rises during the proliferation phase of pregnancy. Cell culture studies with mouse, rat, and human mammary epithelial cells have demonstrated the mitogenic activity of cAMP in the mammary gland.[34,35] cAMP reportedly functions as a cellular regulator of lactogenesis in the mammary gland.[33,34] The addition of cAMP

Table 5 Activity of RNA polymerases in mammary gland in mid- and late pregnancy[1]

Polymerase (cpm/μg of DNA)	Midpregnancy		Late pregnancy		SEM[2]
	Control	Test	Control	Test	
RNA polymerase I	22.4	186.2	331.9	280.0	22.8
RNA polymerase II	533.3	446.8	1168.6	1239.5	48.8
RNA polymerase III	381.0	678.6	199.6	992.2	20.3
Total	936.7	1311.6	1700.1	2511.7	60.4

[1]Determined by the incorporation of [3H]UTP into acini.
[2]Standard error of the mean (SEM), where $n = 16$.

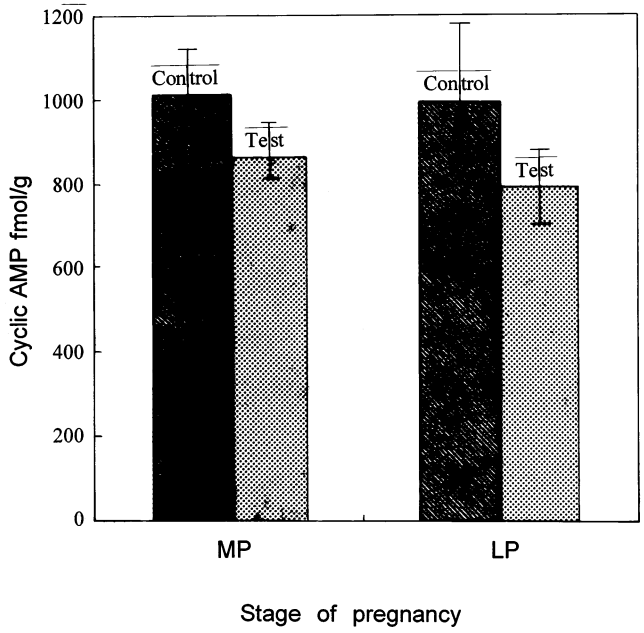

Figure 2 3′,5′-Cyclic adenosine monophosphate (AMP) concentration of mammary tissue. MP, midpregnancy; LP, late pregnancy. Vertical lines represent standard error ($n = 2$).

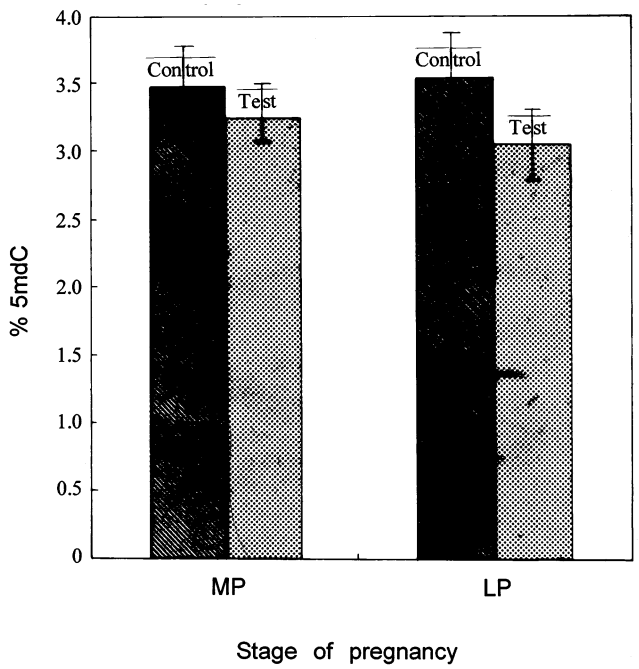

Figure 3 5′-Methyldeoxycytidine (5 mdC) concentration of mammary tissue. MP, midpregnancy; LP late pregnancy. Vertical lines represent standard error ($n = 2$).

to bovine mammary acinar cell culture increased the activity of protein synthesis and amino acid uptake, and these activities were increased in the presence of lactogenic hormones[36]; similar results were obtained in rat mammary epithelial cells.[35]

cAMP functions as a secondary messenger and affects cellular enzyme activation.[37,38] Upon ligand binding, many receptors activate or inhibit adenylate cyclase, which synthesizes cAMP.[37,38] cAMP exerts its effect on cells through the action of cAMP-dependent protein kinases and signals its presence in the nucleus via phosphorylation of the transcription factor, which activates cAMP-inducible gene expression.[38,39] Eventually, this process leads to cell growth or differentiation. In this study, compensatory growth might affect the endocrine system, and such influence might reduce the capacity of the receptors to transfer hormonal signals by the cAMP-dependent pathway, decreasing cAMP levels in mammary glands. Alternatively, compensatory growth may influence the turnover rate of cAMP in the cell. What causes the change of cAMP levels in mammary cells, and which receptor plays a major role in the change of cAMP, remains to be studied.

DNA methylation

The 5 mdC levels in midpregnancy and late pregnancy in the control group was slightly higher than in the test group (*Figure 3*). DNA methylation was lower in the test group than in the control group for all experimental periods.

5 mdC is a minor nucleoside encountered in the DNA of all animals. 5 mdC constitutes 2% to 8% of the total cytidine in animal DNA and is found predominantly in the CG dinucleotides.[40] Changes in DNA methylation occur in many basic processes of eukaryotic cells, including embryogenesis, differentiation, aging, and carcinogenesis.[41,42] Methylation of DNA may exert a controlling influence over gene expression. In most reports concerning DNA methylation and gene activity, a correlation between hypomethylation, in particular in the 5′-flanking region of genes, and gene activity has been demonstrated.[43,44] In our experiment, physiologic change by compensatory growth may have decreased DNA methylation in mammary glands of pregnant heifers. Lower levels of DNA methylation in the test group may be related to the expression of gene in mammary gland.

Acknowledgments

This research was supported by Basic Research Grants, Korea Science and Engineering Foundation, Daejon, Korea (KOSEF 90-05-00-05). The authors thank A.G. Mackinlay, University of New South Wales, Kensington, Australia, for the kind gift of α_{s1}- and β-casein cDNA.

References

1 Park, C.S., Choi, Y.J., Erickson, G.M., and Marx, G.D. (1987). Effect of compensatory growth regulation of growth and lactation: Response of dairy heifers to a stair-step growth pattern. *J. Anim. Sci.* **64,** 1751

2 Grigor, M.R., Allan, J.E., Carrington, J.M., Carne, A., Genrsen, A.R., Young, D., Thompson, M.P., Haynes, E.B., and Coleman, R.A. (1987). Effect of dietary protein and food restriction on milk production and composition, maternal tissues and enzymes in lactating rats. *J. Nutr.* **117,** 1274

3 Park, C.S., Baik, M.G., Keller, W.L., Berg, I.E., and Erickson, G.M. (1989). Role of compensatory growth in lactation: A stair-step nutrient regimen modulates differentiation and lactation of bovine mammary gland. *Growth Dev. Aging* **53,** 159

4 Sejrsen, K., Huber, J.T., Tucker, H.A., and Akers, R.M. (1982).

Influence of nutrition on mammary development in pre- and post-pubertal heifers. *J. Dairy Sci.* **65,** 793
5 Blum, J.W., Schnyder, W., Kunz, P.L., Blom, A.K., Bickel, M., and Schurch, A. (1985). Reduced and compensatory growth: Endocrine and metabolic changes during food restriction and refeeding in steers. *J. Nutr.* **115,** 417
6 Thomsom, E.F., Bickel, H., and Schürch, A. (1982). Growth performance and metabolic changes in lambs and steers after mild nutritional restriction. *J. Agric. Sci.* (Camb.) **98,** 183
7 Wilson, P.N. and Osbourn, D.F. (1960). Compensatory growth after under-nutrition in mammals and birds. *Biol. Rev.* **35,** 324
8 Park, C.S., Choi, Y.J., Keller, W.L., and Harrold, R.L. (1988). Effect of compensatory growth on milk protein gene expression and mammary differentiation. *FASEB J.* **2,** 2619
9 Baik, M.G. (1992). Nutritional modulation of mammary development and differentiation and aging. PhD Dissertation, North Dakota State University, Fargo, ND
10 Suard, Y.M.L., Haeuptle, M.T., Farinon, E., and Kraehenbuhl, J.P. (1983). Cell proliferation and milk protein gene expression in rabbit mammary cell cultures. *J. Cell Biol.* **96,** 1435
11 National Research Council. (1989). *Nutrient Requirements of Dairy Cattle*, 6th ed. National Academy Press, Washington, DC, USA
12 Caruolo, E.V. (1980). Scanning microscope visualization of the mammary gland secretory unit of myoepithelial cells. *J. Dairy Sci.* **63,** 1987
13 Raber, J.M. and D'Ambrosio, S.M. (1986). Isolation of single cell suspensions from the rat mammary gland: Separation, characterization, and primary culture of various cell population. *In Vitro Cell. Dev. Biol.* **22,** 429
14 Smith, J.J., Nickerson, S.C., and Keenan, T.W. (1982). Metabolic energy and cytoskeletal requirements for synthesis and secretion by acini from rat mammary gland. 1. Ultrastructural and biochemical aspects of synthesis and release of milk proteins. *Int. J. Biochem.* **14,** 87
15 Choi, Y.J. (1987). The regulation of mammary differentiation and milk protein gene expression. PhD Dissertation North Dakota State University, Fargo, ND
16 Chomzysky, P. and Sacchi, N. (1987). Single step method of RNA isolation by acid guanidinium thiocynate-phenol-chloroform extraction. *Anal. Biochem.* **162,** 156–159
17 White, B.A. and Bancroft, F.C. (1982). Cytoplasmic dot hybridization: Simple analysis of relative mRNA levels in multiple small cell or tissue sample. *J. Biol. Chem.* **257,** 8569
18 Weil, P.A., Sidikaro, J., Stancel, G.M., and Blatti, S.P. (1977). Hormonal control of transcription in the rat uterus. *J. Biol. Chem.* **252,** 1092–1098
19 Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). *Molecular Cloning*, 2nd ed. Cold Spring Harbor Laboratory Press, New York, NY, USA
20 SAS Institute Inc. (1991). *SAS/STAT User's Guide.* Release 6.03 edition. SAS Institute Inc., Cary, NC, USA
21 Choi, Y.J., Kim, S.H., Cho, K.K., and Han, I.K. (1992). The regulation of mammary development and milk protein gene expression by incremental growth pattern in rats. *Korean J. Anim. Nutr. Feedst.* **16(4),** 183
22 Choi, Y.J., Park, C.S., Keller, W.L., Harrold, R.L., and Haugse, C.N. (1987). Compensatory growth modulates milk protein gene expression. *J. Anim. Sci.* **65 (Suppl. 1),** 254 (Abstr.)
23 Sejrsen, K., Huber, J.T., and Tucker, H.A. (1983). Influence of amount fed on hormone concentrations and their relationship to mammary growth in heifers. *J. Dairy Sci.* **66,** 845
24 Swanson, E.W. (1978). Heifer performance standards: Relation of rearing systems to lactation. In *Large Dairy Herd Management* (H.H. Van Horn and C.J. Wilcox, eds.), pp 494, American Dairy Scientific Association, Champaign, IL, USA
25 Swanson, E.W. and Poffenbarger, J.I. (1979). Mammary gland development of dairy heifers during their first gestation. *J. Dairy Sci.* **62,** 702
26 Johnson, I.D. and Hart, I.C. (1985). Pre-pubertal mammogenesis in the sheep. 1. The effects of level of nutrition on growth and mammary development in female lambs. *Anim. Prod.* **41,** 323
27 Park, C.S. and Bissel, M.J. (1986). Messenger RNA for basement membrane component in the mouse mammary gland and cells in culture. *J. Cell Biol.* **103,** 101a (Proc.)
28 Reeder, R.H. and Roeder, R.G. (1972). Ribosomal RNA synthesis in isolated nuclei. *J. Molec. Biol.* **67,** 433–44441
29 Geiduschek, E.P. and Tocchini-Valentini, G.P. (1988). Transcription by RNA polymerase III. *Ann. Rev. Biochem.* **57,** 873–914
30 Young, R.A. (1991). RNA polymerase II. *Ann. Rev. Biochem.* **60,** 689–715
31 Guyette, W.A., Matusik, R.J., and Rosen, J.M. (1979). Prolactin-mediated transcriptional and post-transcriptional control of casein gene expression. *Cell* **17,** 1013–1023
32 Loizzi, R.F. (1987). Cyclic-AMP changes in guinea pig mammary gland and milk. *Am. J. Physiol.* **8,** 549
33 Sapag-Hagar, M. and Greenbaum, A.L. (1974). The role of cyclic nucleotides in the development and function of rat mammary tissue. *FEBS Lett.* **46,** 180
34 Perry, J.W. and Oka, T. (1980). Cyclic AMP as a negative regulator of hormonally-induced lactogenesis in mouse mammary gland organ culture. *Proc. Natl. Acad. Sci. USA* **77,** 2093
35 Ethier, S.P., van de Velde, R.M., and Cundiff, K.C. (1989). cAMP Levels in proliferating rat mammary epithelial cells in vitro and in vivo. *Exp. Cell Res.* **182,** 653
36 Roh, S.G., Woo, J.H., Baik, M.G., and Choi, Y.J. (1993). Non-hormonal factors on differentiation of bovine mammary cells. *In Vitro Cell. Dev. Biol.* **30A,** 292
37 Neer, E.J. and Clapham, D.E. (1988). Roles of G protein subunits in transmembrane signaling. *Nature* **333,** 129–134
38 Ullrich, A. and Schlessinger, J. (1990). Signal transduction by receptors with tyrosine kinase activity. *Cell* **61,** 203–212
39 Van der Geer, P., Hunter, T., and Lindberg, R.A. (1994). Receptor protein-tyrosine kinases and their signal transduction pathways. *Ann. Rev. Cell Biol.* **10,** 251–337
40 Ehrlich, M., Gama-Sosa, M.A., Huang, L.H., Midgett, R.H., Kuo, K.C., McCune, R.A., and Genrke, C. (1982). Amount and distribution of 5-methylcytosine in human DNA from different types of tissues or cells. *Nucleic Acids Res.* **10,** 2709–2712
41 Wilson, V.L., Smith, R.A., Ma, S., and Cutler, R.G. (1987). Genomic-methyl-deoxycytidine decreases with age. *J. Biol. Chem.* **262,** 9948
42 Mays-Hoopes, L.L. (1989). Age-related changes in DNA methylation: Do they represent continued developmental changes? *Int. Rev. Cytol.* **114,** 181
43 Razin, A., Feldmesser, E., Kafri, T., and Szyf, M. (1985). Cell specific DNA methylation patterns: Formation and a nucleosome locking model for their function. In *Biochemistry and Biology of DNA Methylation* (G.L. Cantoni and A. Razin, eds.), pp 299, Alan R. Liss, New York, NY, USA
44 Doefler, W. (1983). DNA methylation and gene activity. *Ann. Rev. Biochem.* **52,** 93–124