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Effects of a Stair-step Growth Pattern on Improvements in Meat Quality and Growth in Hanwoo Steers*

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ABSTRACT : The present study was conducted to examine the effect of a stair-stepped feed intake pattern on growth, feed efficiency, and meat quality of Hanwoo steers. Twenty-seven 11-month-old Hanwoo steers were randomly divided into three groups. The control group was fed according to the Korean steer feeding program, and the other two groups were fed according to an alternated feeding schedule of 3-2-4-2 months. During the first three months of the experiment, treatment group 1 (T1) and treatment group 2 (T2) were fed 20% and 30% less than the control group, respectively. For the following two months, the T1 group was fed 20% more than the control group while the T2 group was fed 20% less than the control group. In the third step, T1 and T2 groups were fed 20% and 10% less, respectively, than the control group for four months. In the last two months, T1 and T2 groups were fed 20% more than the control group. After the stair-step feeding trial, steers were fed concentrated feed *ad libitum* for five months. The altered feed intake pattern did not affect daily body weight gain. However, daily feed intake tended to decrease and growth efficiency tended to increase in the two treatment groups compared to the control group. Altered feed intake also affected blood metabolite levels. The serum glucose and BUN level of the T1 group increased in the first re-fed period compared to the T2 and control groups. The serum cholesterol level of the T2 group decreased in the first restricted-re-fed growth period compared to the T1 and control groups. The serum NEFA levels of the two treatment groups increased from the first restricted period compared to the controls. The serum insulin level of the T2 group increased in the last period compared to the T1 and control groups. Regarding meat yield index, the control group was significantly higher than the T2 group ($p<0.05$). Regarding meat yield grade, the carcass back fat thickness of the T2 group was significantly higher than the control group ($p<0.05$). In marbling score, the T1 group was the highest (4.9), followed by the control group (4.1) and the T2 group (4.0). These results indicate that using a stair-stepped growth pattern (T1) can contribute to improvements in growth efficiency and muscle marbling.
(**Key Words :** Stair-step Growth Pattern, Growth Efficiency, Blood metabolites, Marbling Score, Hanwoo)

INTRODUCTION

In general, compensatory growth occurs when previously marginal- or under-fed animals are re-alimented onto a higher nutritional level. Compensatory growth following a period of malnutrition depends on the nature of the restricted diet, the severity and duration of malnutrition, the stage of development at the outset of malnutrition, the relative mature body weight (BW) of the animal, and the pattern of realimentation (Wilson and Osbourn, 1960; Abdalla et al., 1988; Jin et al., 2004).

A number of investigators have reported compensatory growth responses with rats (Harris and Widdowson, 1978; Park et al., 1988; Choi et al., 1992; Park et al., 1994), pigs (Sarkar et al., 1983; Ahn et al., 1996), sheep (McManus, 1972), broilers (Pokniak and Cornejo, 1982), rabbits (Ledin, 1984), gilts (Crenshaw et al., 1989a; Crenshaw et al., 1989b), beef cattle (Bohman, 1955; Koch, 1982; Nelsen et al., 1982; Choi et al., 1996; Jin et al., 2004), and dairy heifers (Park et al., 1987; Park et al., 1989; Jang et al.,

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1995; Choi et al., 1997; Park et al., 1998). During compensatory growth, animals show great increases in body weight, increased efficiency of energy utilization, reduced maintenance requirements due to basic metabolic rate depression, enhanced appetite and feed intake capacity, changes in endocrine status, altered body tissue composition, increased mammary growth, and differentiation and performance at subsequent lactations compared with animals fed conventional diets (Crenshaw et al., 1989a; Crenshaw et al., 1989b; Choi et al., 1992; Park et al., 1994; Jang et al., 1995; Ahn et al., 1996; Choi et al., 1996; Choi et al., 1997; Park et al., 1998). However, there are conflicting opinions regarding the physiological basis of compensatory growth in beef cattle.

Therefore this study was conducted to examine the effect of compensatory growth feeding on growth, feed efficiency, and meat quality of Korean native cattle.

MATERIALS AND METHODS

Animals and diets

Twenty-seven, 11-month-old Hanwoo steers were divided into three groups of nine animals each based on body weight (293±22.8 kg). This trial was conducted over a period of 16 months. To avoid metabolic disease due to excessive concentrate feeding during the stair-step growth period, these steers were put through a two month adaptation period. During the experimental period, steers were fed twice daily and had free access to water and red mineral blocks. Control group steers were fed according to the Korean steer feeding program, and treatment groups were subjected to a stair-step nutrition scheme according to an alternating schedule of 3-2-4-2 months. Treatment group 1 (T1) steers were fed 20% less than the control group in the first three months of the trial, 20% more than the control group for two months, 20% less than the control group for four months, and 20% more than the control group for two months. Treatment group 2 (T2) animals were fed 30% less than the conventional diet for three months, followed by 20% less than the control group for two months, 10% less than the control group for four months, and 20% more than the control group for two months. After the stair-step feeding trial, steers were again fed concentrate *ad libitum*, along with 1 kg hay per day (dry matter). The roughage was mainly composed of *borme* hay. Experimental feeds were analyzed for DM (Dry matter), Crude fiber, CP (Crude protein), EE (Ether extract), Ca and P by the methods of AOAC (1995). The ingredients and chemical composition of the experimental diets are shown in Table 1.

Real-time ultrasound measure

Real-time ultrasound measurements were obtained from the Scanner unit (2MHz linear, Super-Eye Meat, FHK, Japan). A single image by this equipment of scanned 13th rib rump was interpreted by video copy processor (AP-950, Mitsubishi, Japan) and computer program (Image-Pro Express, Media Cybernetics, USA) (Rhee et al., 2005).

Measurements of feed intake and body weight

During the experiment, body weights of the steers were measured monthly, and steers were fed pre-weighed concentrate feed at 8 am and 5 pm every day. The amount of concentrate feed left uneaten by the steers was weighed one hour prior to the next day's morning feeding.

Blood sample collection and analysis

During the experiment, blood samples were collected a total of five times from the jugular vein of each steer prior

Table 1. Ingredients and chemical composition of the experimental diets

Variable	Control		T1		T2	
	Roughage[1]	Concentrate[2]	Roughage	Concentrate	Roughage	Concentrate
DMI (kg/d)						
0-3 months	3.00	6.00	3.00	4.45	3.00	3.75
3-5 months	2.50	8.50	2.15	10.81	2.65	6.43
5-9 months	1.75	10.00	1.75	7.73	1.75	8.94
9-11 months	1.10	10.00	1.55	11.85	1.55	11.85
Chemical analysis (% of DM)						
CP	5.84	12.00				
Crude fat	0.67	2.50				
Crude fiber	29.80	15.00				
Ca	0.14	0.70				
P	0.10	0.40				
TDN	0.47	0.72				

[1] The roughage was mainly composed of *brome* hey. [2] The concentrate was produced by WooSung Feed. Co. Ltd.
T1: stair-step growth pattern; T2: restricted - re-fed.
DMI = Dry matter intake; CP = Crude protein; TDN = Total digestible nutrients.

Table 2. Primer sequences for real-time PCR of specific genes

Gene	Primer nucleotide sequence	Product size (bp)
β-GAPDH	Forward 5' GAT CCT GCC AAC ATC AAG TG	137
	Reverse 5' AGC AGA AGG TGC AGA GAT GA	
C/EBP-a	Forward 5' CAA GAA GTC CGT GGA CAA GA	142
	Reverse 5' ATT GTC ACT GGT CAG CTC CA	
aP2	Forward 5' GGG TGT GGT CAC CAT TAA AT	113
	Reverse 5' CGA TGC TCT TGA CTT TCC TG	
ACC	Forward 5' CAT ATC GCA TCA CAA TTG GC	87
	Reverse 5' TTA ACT TCC CAG CAG AGG GT	
FAS	Forward 5' GAC AGA GCA CGC CTT CAT AA	139
	Reverse 5' GGA AGT TGA GGG AGG CAT AA	
SCD	Forward 5' TTC TTC TCT CAC GTG GGT TG	118
	Reverse 5' ACC TCC TCT GGA ACA TCA CC	

aP2: adipocyte fatty-acid-binding protein, SCD: stearoyl-coenzyme A desaturase.
FAS: fatty acid synthase, ACC: acetyl-CoA Carboxylase.

to the morning feeding (0, 3, 5, 9, and 11 months after experiment start). Blood samples were centrifuged at 3,000 rpm for 15 minutes to obtain serum, which was stored at -70°C until analysis. The levels of serum glucose, BUN, and total cholesterol were measured using a TBA-40FR automated biochemical analyzer (Toshiba, Japan) according to Lee et al. (2005). Serum NEFA level was measured using a Wako Pure Chemical Industries (Osaka, Japan) commercial kit, and serum insulin level was measured using a bovine-specific insulin ELISA kit (Mercodia co, Sweden).

Analysis of real-time polymerase chain reaction (PCR)

The real time-PCR technique was used to measure mRNA expression levels for aP2, C/EBP-a, ACC, FAS, and SCD. GAPDH was used as an invariant internal control. Information about primer sequences of the specific genes for real time-PCR is given in Table 2. Total RNA was isolated from the subcutaneous fat and *longissimus* muscle using Trizol reagent (Invitrogen Life Technologies, USA). PCR reactions were begun at 95°C for three minutes, followed by 50 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s. The relative quantification of gene expression was analyzed using the $2^{-\Delta\Delta CT}$ method (Livak et al., 2001).

Statistical analysis

The basic statistical model for the stair-step growth regimen has been described previously (Park et al., 1989). The experimental data were expressed as mean±SEM. Data were analyzed using SPSS software (Statistical Package for the Social Sciences; version 14.0, SSPS Inc, Chicago, USA), and treatment effects were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range test. A p value <0.05 was considered to be statistically significant.

RESULT AND DISCUSSION

Growth efficiency and feed efficiency

After the two-month preliminary feeding experiment, restricted feeding on restricted - re-fed growth, early fattening for 3-2-4-2 months, and finally late fattening and feeding, the results for growing/fattening weight grades are shown in Figure 1 and Table 3. For the first restricted - re-fed growth period, the daily body weight gains of the control and T1 groups showed no significant difference, but that of the T2 group increased significantly ($p<0.01$). Regarding feed intake, the control group was significantly higher ($p<0.05$) than the T1 and T2 groups, but T1 was greater than T2 ($p<0.05$). Regarding growth efficiency, the T1 group was the highest with an efficiency of 9.73%, while the control group was 9.17% and the T2 group was 8.63%. During the second restricted - re-fed growth period, daily body weight gain of T2 increased significantly ($p<0.01$) compared to T1; for feed intake, T2 was the highest at 9.80 kg/d, followed by the control at 9.76 kg/d and T1 at 9.16 kg/d. Regarding feed efficiency, there was no

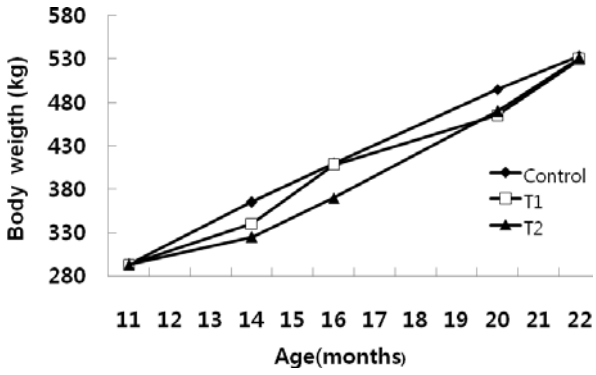

Figure 1. Growth curves of experimental Hanwoo steers. Stair-step growth (3-2-4-2 month scheme). T1: stair-step growth pattern, T2: restricted - re-fed growth pattern.

Table 3. Production performance during two stair-step growth feeding patterns

Item	Control	T1	T2
Period 1 (0-5 months)			
Daily gain (kg)	0.79±0.05[a]	0.78±0.03[a]	0.63±0.02[c]
Feed intake (DM kg/d)	8.67±0.17[a]	8.02±0.21[bc]	7.35±0.21[c]
Growth efficiency (%)	9.17±0.55	9.71±0.44	8.63±0.31
Period 2 (5-11 months)			
Daily gain (kg)	0.64±0.04[c]	0.65±0.03[c]	0.78±0.02[a]
Feed intake (DM kg/d)	9.76±0.26	9.16±0.30	9.80±0.28
Growth efficiency (%)	6.50±0.37[c]	7.13±0.28[ac]	8.06±0.37[a]
Ad libitum period (11-16 months)			
Daily gain (kg)	0.76±0.04	0.84±0.02	0.90±0.01
Feed intake (DM kg/d)	9.31±0.31	9.58±0.41	10.44±0.37
Growth efficiency (%)	8.16±0.35	8.57±0.42	8.63±0.39
Total period (0-11 months)			
Daily gain (kg)	0.71±0.04	0.71±0.02	0.71±0.01
Feed intake (DM kg/d)	9.30±0.21	8.67±0.26	8.72±0.25
Growth efficiency (%)	7.63±0.42	8.21±0.25	8.27±0.31

Data are mean±SEM of 9 steers.
Efficiency: (daily weight gain/daily feed intake)×100%; DM = Dry matter.
T1: stair-step growth pattern; T2: restricted - re-fed growth pattern.
Significance of differences among the control, T1 and T2 groups; [a, b] $p<0.05$; [a, c] $p<0.01$.

significant difference between T2 and T1, but both showed a higher feed efficiency than the control ($p<0.01$). Throughout the two restricted - re-fed growth periods, the daily body weight gain was similar between the treated groups, but feed intake, was 9.3 kg/d for the control, 8.72 kg/d for T2 and 8.67 kg/d for T1. For growth efficiency, T2 increased the most at 8.27%, T1 increased 8.21%, and the control increased 7.63%. These results were consistent with the results of stair-step growth feeding in which less feed intake results in increased feed efficiency and growth ability, possibly due to the promotion of efficient energy and protein use. Fasting animals had further enhanced weight gain upon re-fed supply compared with non-fasting animals fed the same feed, which is partially due to the reduction of maintenance requirements and increased feed use. This result is consistent with the results of stair-step growth feeding experiments with Holstein heifers during a feed-restricted feeding period, in which weight gain decreased, but during the re-fed growth period, the growth efficiency, energy, and protein use efficiency were greatly increased (Koch, 1982; Blum et al., 1985; Park et al., 1987; Park et al., 1989; Drouillard, 1990; Choi et al., 1992; Ryan et al., 1993a; Ryan et al., 1993b; Park et al., 1994; Jang et al., 1995; Ahn et al., 1996; Choi et al., 1996; Choi et al., 1997; Park et al., 1998). In addition, according to Hornick et al. (2000), growth hormone was secreted during the maintenance period and was maintained at high concentration in the re-fed growth period; also the increased secretion of insulin due to increased nutrient intake and collaboration of mass action showed re-fed growth effects.

Changes in blood metabolites and insulin

During two restricted - re-fed growth experiment periods, the blood metabolite changes shown in Figure 2 were observed. The level of blood glucose (Figure 2A) in the first re-fed growth period of the T1 group increased significantly compared to the control and T2 groups. In addition, during the entire experiment the T1 group showed higher blood glucose levels than T2; after the re-fed growth period, the blood glucose level of T2 was higher than the control group. This result is similar to that of Park et al. (1987) for the stair-step growth of Holstein fattening. Furthermore, the blood concentration of glucose has a tendency to decrease with increased age. According to Stufflebeam et al. (1969), in an experiment using 58-week-old heifers which were divided into three groups of 16 depending on the level of energy supply and fed an experimental diet for 224 days, blood glucose concentrations decreased gradually with growth but did not have any relationship with energy intake.

For BUN (Figure 2B), the T1 group maintained a low level during the feeding period, and showed a rapid increase during the re-fed growth period ($p<0.05$). However, during the two maintained feeding periods, a rapid decline in BUN concentration was noted, which increased significantly in the re-fed growth period compared to the control group ($p<0.05$). The results of some studies (Park et al., 1987; Choi et al., 1996) are similar to the low level observed herein during the feeding period, but increased sharply in the re-fed growth period. Huntington et al. (1996) showed that as the combination of feed supply increased, BUN

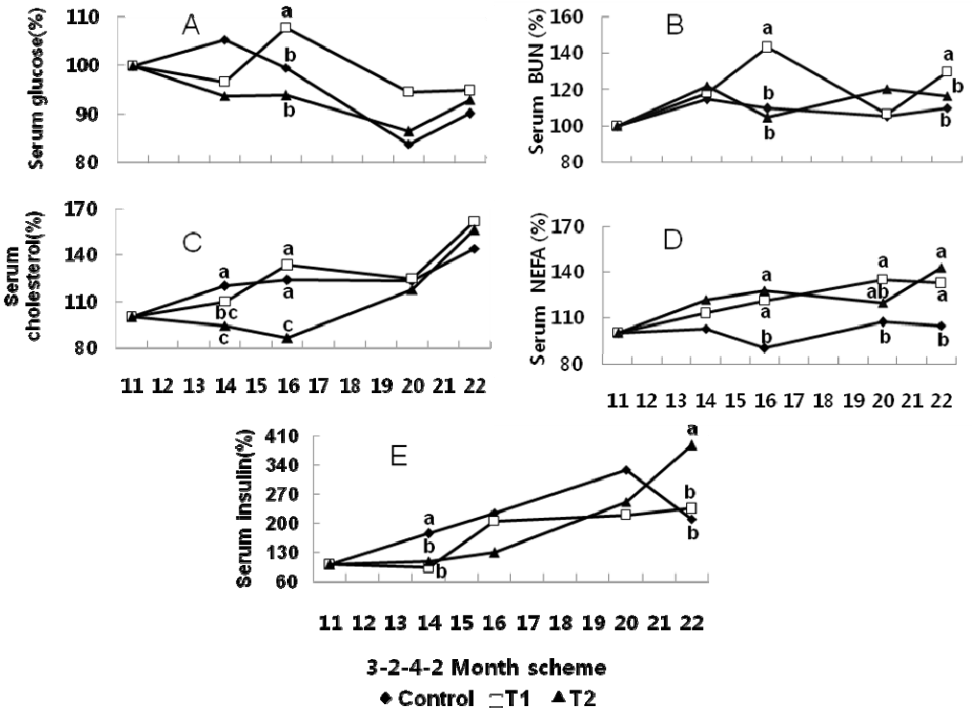

Figure 2. Blood serum levels of glucose, BUN (blood urea-nitrogen), total cholesterol, NEFA (non-esterified fatty acids), and insulin during two stair-step growth patterns. Values are expressed as percentage of the initial level**; s**ignificance of differences among the control, T1 and T2 groups. a, b: $p<0.05$; a, c: $p<0.01$. T1: stair-step growth; T2: restricted - re-fed growth.

concentration also increased. Moreover, Philippe et al. (2005) showed that the growth of fatty tissue increased significantly outside of the muscle tissue during the re-fed growth period. Based on these results, the storage capacity of fat tissue is considered higher than the protein synthesis capacity while using the energy provided in the re-fed growth period. Therefore, animals experiencing re-fed growth take in an excessive amount of feed yielding a fat synthesis rate higher than that of protein synthesis, upsetting the body's energy and protein balances. Increased ammonia produced during metabolism is converted to urea in the liver causing an increased BUN concentration.

The total blood cholesterol density (Figure 2C) of the control group increased significantly compared to T1 and T2 groups during the restricted feeding period ($p<0.05$, $p<0.01$, respectively). However, T1 and control groups showed no significant difference during the re-fed growth period, which is consistent with the results of Park et al. (1988). Furthermore, Arave et al. (1975) reported that as growth and feed energy intake increased, blood cholesterol levels also increased. In addition, total blood cholesterol increased with animal age, but decreased with high content of protein in feed (Park et al., 1980, 1983). In this experiment, during the two maintenance re-fed periods there was no significant difference between the treated groups. The T1 and T2 groups had higher cholesterol concentrations in their blood due to excessive feed intake compared to the control group.

From the changes in serum NEFA concentration (Figure 2D) during the first re-fed period, T1 and T2 groups showed and maintained higher values than the control group ($p<0.05$), which was consistent with the results of Blum et al. (1985). In addition, Yambayamba et al. (1996) showed that in the maintenance period serum NEFA concentration of heifers increased significantly, but with increased feed intake the NEFA concentration decreased. Hornick et al. (2000) reported that during the restricted feeding period increased secretion of growth hormone promoted breakdown of fat to maintain body energy balance, which then increased the serum NEFA concentration. In the current experiment, however, the rapidly increasing energy intake during the re-fed growth period also increased blood NEFA concentrations that were maintained at high level regardless of the reduction in energy intake. Emery (1979) showed that in the ruminant body fat accumulation was mainly due to accumulation of triglycerides and NEFA in blood in dynamic equilibrium. Pothoven et al. (1975) reported that fatty acid-mobilizing lipase activity in fat tissue increased as fattening progressed. Therefore, in our experiment, during the second maintenance re-fed period the serum NEFA concentration increased.

Changes in blood concentration of insulin are shown in Figure 2E. During the first maintenance growth feed period, blood insulin concentrations of T1 and T2 groups decreased

Table 4. Early prediction of marbling score by ultrasound in Hanwoo steers

Items	Age (months)					
	24			28 (slaughter)		
	Control	T1	T2	Control	T1	T2
Body weight (kg)	612.0±24.5[1]	599.0±3.3	620.0±8.6	667.4±25.5	680.3±13.2	692.6±9.6
Marbling score	4.1±0.8	4.7±0.5	3.9±0.6	4.1±0.6	4.9±0.6	4.0±0.8
Back fat thickness (mm)	8.1±0.8[a]	4.9±0.5[b]	9.6±0.7[a]	11.9±1.9[b]	11.1±1.4[ab]	16.9±2.2[a]

T1: Stair-step growth; T2: Restricted - re-fed growth. [1] Mean±SEM of 9 steers.
[a, b] Means with different superscripts in the same column differ significantly ($p<0.05$).

compared to the control group ($p<0.05$), and in the re-fed supply period insulin concentration tended to increase in T1 and T2. During the second re-fed growth period, the insulin level of the T2 group was significantly increased compared to the control and T1 groups ($p<0.05$). The insulin level of the T1 group showed a tendency to increase compared to the control. Insulin concentration is known to have a correlation with feed or energy intakes (Bassett, 1972). Hornick et al. (2000) reported that increased feed intake during the re-fed growth period resulted in a sudden increase of insulin concentration in the blood. Jang et al. (1995) reported that during stair-step growth feeding of Holstein cattle, plasma insulin concentrations were lower than in the control group, but higher concentrations were noted during re-fed growth; Blum et al. (1985) reported a similar result. The results of the present experiment agree with previous reports. This indicates that during the maintenance period insulin secretion decreases, but as nutrient intake and feed intake are increased insulin secretion also increases and promotes the synthesis of protein and fat tissues.

Rhee et al. (2005) reported that, by using an early prediction system with ultrasound, flesh and meat yields could be evaluated. Using ultrasound in this experiment, we performed early prediction on 24-month-old Korean native steers. The evaluation results are shown in Table 4; marbling score was highest in the T1 group, followed by the control and T2 groups. For back fat thickness, the T1 group was significantly lower than T2. After slaughter at 28 months, the marbling score of the T1 treated group was higher than those of the control and T2 groups. For back fat thickness, T1 showed no significant difference with either the control or T2 groups, while T2 was significantly higher than the control group. According to these results, animals that experienced multiple re-fed growth periods had an increased ability to synthesize fat than did the control and T2 groups in the early fattening phase; however these same animals had a decreased ability for synthesis of subcutaneous fat. Fattening, according to the marbling score, increased in the treated groups, but the back fat thickness of T1 and T2 increased more than that of the control group ($p<0.05$). These results are consistent with the results of

Table 5. Carcass characteristics of Hanwoo steers by treatment

Item	Control	T1	T2
Yield grade			
Fasting body wt. (kg)	667.4±25.5[1]	680.3±13.2	692.6±9.6
Cold carcass wt. (kg)	391.3±18.2	397.1±9.6	406.7±9.3
Dressing (%)	58.6±0.1	58.3±0.1	58.7±0.1
Rib-eye area (cm^2)	93.8±2.3	89.7±2.1	90.8±3.1
Fat thickness (mm)	11.9±1.9[b]	11.1±1.4[ab]	16.9±2.2[a]
Meat yield index	67.3±1.4[a]	66.1±0.9[ab]	62.9±1.5[b]
Grade (A:B:C, head)	6 :2:1	4:5:0	1: 5: 3
Quality grade[2]			
Marbling score	4.1±0.6	4.9±0.6	4.0±0.78
Meat color	5.0	5.0	5.0
Fat color	3.0	3.0	3.0
Firmness	1.3±0.2	1.2±0.2	1.6±0.2
Maturity	2.0	2.0	2.0
Grade (1^{++}:1^{+}:1:2:3)	0:2:4:2:1	1:2:4:2:0	1:2:1:4:1

[a, b] Means with different superscripts in the same column differ significantly ($p<0.05$).
[1] Means±SEM of 9 steers. [2] Grading ranges are 1 to 7 for marbling score with higher numbers for better quality, and 1 to 7 for meat and fat colors, 1 to 3 for firmness, maturity and grade with lower numbers of better quality.
T1: stair-step growth pattern, T2: restricted - re-fed.

Dulloo et al. (2002) who found that in the re-fed growth period the body weight gain from fat tissue increased more than the body weight gain from muscle tissue. This result is due to the body's reaction to favour nutrient storage to help assure survival in periods of malnutrition.

Carcass characteristics of experimental cattle of each treated group are shown in Table 5. In T2 and T1 groups these were higher than for the control group. For meat yield grade and carcass back fat thickness, the T2 group scored significantly higher than the control ($p<0.05$), but the T1 stair-step treated group did not show a significant difference. For meat yield index, the control group was significantly higher than the T2 group ($p<0.05$), while the T1 group did not show a significant difference. For Grade A scoring, the control group was the highest at 66.7%, followed by T1 at 44.4%, and T2 at 11.1%. For marbling score, which is the most important determinant of flesh grade, the T1 group had the highest (4.9), followed by the control (4.1), and T2 (4.0) groups. According to the results, during late fattening the T2 group increased in daily body weight gain and feed efficiency compared to T1 and control groups; fat synthesized by the re-fed growth effect was mainly stored as subcutaneous fat resulting in a lower meat yield grade.

Changes in fat metabolism-related gene expression

For sirloin and back fat tissue of Korean native steers, the results of fat cell differentiation and fat synthesis gene expression are shown in Table 6. Back fat tissue and the sirloin quality of beef are important factors which determine meat quality. Marbling score is one very important factor for determining the grade of beef in the United States (USDA, 1989), Japan (JMGA, 1988), and Korea. The growth of fat tissue is determined by the differentiation and size of fat cells. CCAAT/enhancer binding protein (C/EBPs) belongs to the basic-leucine zipper transcription factor family and with PPARrγ, is known to perform important functions in the expression of genes such as ap2 and the terminal differentiation of fat precursor cells (Grimaldi, 2001). In this experiment, C/EBP-a mRNA expression level in muscle tissue of the T1 group was higher than that of the T2 or control groups. In back fat tissue, the T1 group showed lower levels than the control group and significantly lower levels than the T2 group ($p<0.05$). These results showed that differentiation of fat cells in muscle tissue of the T1 group was more active than in the control and T2 groups; on the contrary, that of the back fat cell in the T1 group was less active than the control and T2 groups. Adipocyte protein 2 (ap2) is called the adipocyte fatty acid-binding protein and is an important transcription factor in the terminal differentiation stage of fat precursor cells, which is controlled by PPARγ and C/EBPa (Macdougald and Lane, 1995). Separated fat synthesized by the liver and absorbed from feed is moved within muscle and fat cells (Sul and Dong, 1998). Because in these experiments we analyzed the gene expression level of ap2, we also analyzed the use of late fattening energy sources. The result was that aP2 mRNA expression in muscle tissue of the T1 group was higher than that of the control and T2 groups, while in fat tissue, T2 and T1 groups showed greater aP2 expression than the control group. These results showed that the use of separated fatty acid by muscle and fat cells was increased in the T1 group, and separated fatty acid within back fat tissue was increased in the T2 group. Acetyl-CoA formed through β-oxidation in mitochondria acts as a precursor of fat synthesis, which is converted into malonyl-CoA by acety-CoA carboxylasea (ACC). Finally, palmitate is synthesized as a long chain fatty acid form by fatty acid synthase (FAS). Synthesized long chain fatty acid is converted into monounsaturated fatty acid by stearoyl-CoA desaturase and accumulates in muscle and fat tissue as triglycerides (Sul and Dong, 1998).

In muscle and fat tissues, acetyl-CoA carboxylase (ACC) is known as a key enzyme to control fat synthesis rate. *De novo* lipogenesis begins with the carboxylation of acetyl-CoA and its conversion into malonyl-CoA (Murray et al., 2000). The ACC gene is known to react very sensitively to nutrition level. In this experiment, the ACC level of the T1 group was higher than that of the control and T2 groups, not only in muscle tissue but also in back fat tissue, although there was no significant difference. The late

Table 6. mRNA expression in Hanwoo muscle and fat tissues

	Muscle tissue			Back fat tissue		
	Control	T1	T2	Control	T1	T2
C/EBP-a	0.91±0.41[1]	1.14±0.49	0.94±0.53	1.21±0.28[ab]	0.47±0.09[b]	1.87±0.82[a]
aP2	0.97± 0.42	1.05±0.22	0.59±0.23	1.24± 0.33	2.03±1.28	2.20±0.88
ACC	1.18±0.21	1.42±0.30	0.70±0.34	1.71±0.57	2.71±1.43	2.19±1.27
FAS	1.21±0.29	1.39±0.63	0.32±0.10	0.54±0.08	0.60±0.08	0.44±0.16
SCD	1.06±0.28	1.34±0.45	1.02±0.34	2.56±1.45	2.29±1.30	3.36±2.02

T1: stair-step growth pattern; T2: restricted - re-fed growth pattern. [1] Mean±SEM of 9 steers.
[a, b] Means with different superscripts in the same column differ significantly ($p<0.05$).
aP2: adipocyte fatty-acid-binding protein, SCD: stearoyl-coenzyme A desaturase.
FAS = Fatty acid synthase, ACC = Acetyl-CoA Carboxylase.

fattening period of stair-step feeding causes growth of muscle and fat tissue to increase with the induction of ACC gene expression resulting in the accumulation of fat in muscle and subcutaneous fat tissues.

FAS (fatty acid synthase) mRNA expression in muscle as well as back fat tissue was higher in the T1 group than in the control and T2 groups. FAS is an important enzyme controlling the synthesis of fat and is very sensitive to nutritional status. Clarke et al. (1990) showed that when rats were fasting for 1-2 days, fatty acid synthase (FAS) mRNA in rat liver decreased four-fold, while the amount of FAS mRNA increased several times after 48 hours of fasting and re-feeding with a high carbohydrate diet. According to Mariashiara et al. (1995), the FAS mRNA level of rats increased rapidly after 48 hours fasting and re-feeding. Therefore, considering that the T1 group experienced several restricted/re-fed cycles, an increase of FAS mRNA expression upon intake of nutrients at the end of the fattening cycle was observed and facilitated fat synthesis both in muscle and fat tissues.

Stearoyl-CoA desaturase (SCD) is the major enzyme controlling the synthesis of unsaturated fatty acid in higher animals. SCD uses palmitic acid (16:0) and stearic acid (18:0) as substrates to synthesize palmitoleic acid (16:1) and oleic acid (18:1), respectively. Palmitoleic acid (16:1) and oleic acid (18:1) are the major components of neutral fat (triglycerides). In muscle tissue of this experiment, there were no significant differences among the three groups, but the T1 group showed the highest muscle content, while the T2 group showed the most back fat tissue. The re-fed growth effect on the late fattening feed intake of the T2 group yielded significant results. In meat grades, the T2 group showed a significantly higher score than did the T1 group. Upon late fattening, the T2 group developed fat tissue more actively than muscle tissue. Considering these results, in the muscle tissue of the T1 group, the differentiation of fat cells and use of free fatty acid was increased. However, in back fat tissue, differentiation of fat cells decreased, but use of separated fatty acid increased. In the T2 group, the differentiation of fat cells and use of separated fatty acid increased more in fat tissue than in muscle tissue. Even though there was no significant difference in gene expression level of fatty acid synthetic enzymes among the groups, there was an increase in the back fat tissue of the T2 group. This result may be due to the long-term free feed intake of the T1 and control groups, but for the T2 group, high feed intake gave greater results due to the initial feed restrictions.

REFERENCES

Abdalla, H. O., D. G. Fox and M. L. Thonney. 1988. Compensatory gain by Holstein calves after underfeeding protein. J. Anim. Sci. 66:2687-2695.

Ahn, K. H., I. K. Han, K. Kwon, J. H. Woo and Y. J. Choi. 1996. Effects of stair-step feeding pattern by manipulating energy/protein contents on the growth rate, mammary gland development and reproductive performance. Kor. J. Anim. Nutr. Feed. 20:257-268.

AOAC. 1995. Official methods of analysis. 16th Ed. Association of Official Analytical Chemists, Washington DC, USA.

Arave, C. W., R. H. Miller and R. C. Lamb. 1975. Genetic and environmental effects on serum cholesterol of dairy cattle of various ages. J. Dairy Sci. 58:(3):423-427.

Bassett, J. M. 1972. Plasma glucagon concentrations in sheep: their regulation and relation to concentrations of insulin and growth hormone. Aust. J. Biol. Sci. 25:1277-1287.

Blum, J. W., W. Schnyder, P. L. Kunz, A. K. Blom, M. Bickel and A. Schürch. 1985. Reduced and compensatory growth: endocrine and metabolic changes during food restriction and refeeding in steers. J. Nutr. 115(4):417-424.

Bohman, V. R. 1955. Compensatory growth of beef cattle. The effect of hay maturity. J. Anim. Sci. 14:249-255.

Choi, Y. J., S. H. Kim, K. K. Cho and I. K. Han. 1992. The regulation of mammary development and milk protein gene expression by stair-step growth pattern in rats. Kor. J. Anim. Nutr. Feed. 16:183-190.

Choi, Y. J., H. J. Lee and Y. G. Ko. 1996. Effects of stair-step growth pattern on growth efficiency and blood metabolites in Korean cattle. Kor. J. Anim. Nutr. Feed. 20:529-535.

Choi, Y. J., I. K. Han, J. H. Woo, H. J. Lee, K. Jang, K. H. Myung and Y. S. Kim. 1997. Compensatory growth in dairy heifers: The effect of a compensatory growth pattern on growth rate and lactation performance. J. Dairy Sci. 80:519-524.

Clarke, S. D., M. K. Armstrong and D. B. Jump. 1990. Nutritional control of rat liver fatty acid synthase and S14 mRNA abundance. J. Nutr. 120:218-224.

Crenshaw, J. D., C. S. Park, P. M. Swantek, W. L. Keller and R. C. Zimprich. 1989a. Lactation response of gilts to a phased feeding regimen designed to induce compensatory growth. J. Anim. Sci. 67(Suppl. 2):107-108(Abstr.).

Crenshaw, J. D., C. S. Park, P. M. Swantek, M. P. Storlie, W. L. Keller and R. C. Zimprich. 1989b. Growth, reproduction and lactation response of gilts to a phased feeding regimen designed to induce compensatory growth. J. Anim. Sci. 67 (Suppl. 1):228(Abstr.).

Drouillard, J. S. 1990. Changes in visceral organs, body composition and performance of ruminants in response to discontinuous pattern of growth. Ph. D. Diss., Univ. Nebraska, Lincoln.

Dulloo, A. G., J. Jacquet and J-P Montani. 2002. Pathways from weight fluctuations to metabolic diseases: focus on maladaptive thermogenesis during catch-up fat. Int. J. Obes. Relat. Metab. Disord. 26 (Suppl. 2): S46-57.

Emery, R. S. 1979. Deposition, secretion, transport and oxidation of fat in ruminants. J. Anim. Sci. 48(6):1530-1537.

Grimaldi, P. A. 2001. The roles of PPARs in adipocyte differentiation. Prog. Lipid. Res. 40(4):269-281.

Harris, P. M. and E. M. Widdowson. 1978. Deposition of fat in the body of the rat during rehabilitation after early undernutrition. Br. J. Nutr. 39:201-211.

Hornick, J. K., C. Van Eenaeme, O. Gérard, I. Dufrasne and L. Istasse. 2000. Mechanisms of reduced and compensatory growth. Domest. Anim. Endocrinol. 19(2):121-132.

Huntington, G. B., E. J. Zetina, J. M. Whitt and W. Potts. 1996. Effects of dietary concentrate level on nutrient absorption, liver metabolism, and urea kinetics of beef steers fed isonitrogenous and isoenergetic diets. J. Anim. Sci. 68:3086-3095

Jang, K., Y. J. Choi, H. J. Lee, Y. Y. Cho, J. S. Kim, B. S. Ahn, I. K. Han, K. H. Myung and Y. S. Kim. 1995. Effects of stair-step growth pattern on improvement of production in holstein heifers. Kor. J. Anim. Nutr. Feed. 19(2):135-141.

Jin, M. G., H. G. Lee, Z. S. Hong, J. H. Wang, Y. H. Yin, R. H. Jin, K. K. Cho and Y. J. Choi. 2004. Effect of stepped pattern of feed intake using rice straw as roughage source on regulation of growth, reproduction and lactation in dairy heifers. Asian-Aust. J. Anim. Sci. 17(6):794-798.

JMGA. 1988. New beef carcass grading standards. Japan Meat Grading Association, Tokyo, Japan.

Kaestner, K. H., J. M. Ntambi, T. Kelly, Jr. and M. D. Lane. 1989. Differentiation-induced gene expression in 3T3-L1 preadipocytes. A second differentially expressed gene encoding stearoyl-CoA desaturase. J. Biol. Chem. 264(25): 14755-14761.

Koch, A. 1982. A kinetic model for growth as a function of time and nutritional status. Growth 46:74-87.

Ledin, I. 1984. Effect of restricted feeding and realimentation on growth, carcass composition and organ growth during the first seven days of realimentation in rabbit. Acta Agric. Scand. 34:54-66.

Lee, H. G., Y. J. Choi, S. R. Lee, H. Kuwayama, H. Hidari and S. K. You. 2005. Effects of dietary protein and growth hormone-releasing peptide(GHRP-2) on plasma IGF-1 and IGFBPs in Hostein steers. Domest. Anim. Endocrinol. 28:134-146.

Livak, K. J. andT. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time PCR and the 2(-Delta Delta C(T) Method, Methods 25(4):402-408.

MacDougald, O, A. and M. D. Lane. 1995. Transcriptional regulation of gene expression during adipocyte differentiation. Annu. Rev. Biochem. 64:345-373.

Mariashiara, S., S. F. Yet, Y. Moon, J. Y. Chun and H. S. Sul. 1995. Hormonal and nutritional regulation of the fatty acid synthase promoter in transgenic mice. J. Biol. Chem. 270:30339-30343.

McManus, W. R. 1972. Studies of compensatory growth in sheep. J. Agric. Sci. 79:1.

Murray, R. K., D. K. Granner, P. A. Mayes and V. W. Rodwell. 2000. Biosynthesis of fatty acids. In Harper's Biochemistry 25ed, Prentice-Hall, NJ. 230-237.

Nelsen, T. C., C. R. Long and T. C. Cartwright. 1982. Postinflection growth in straightbred and crossbred cattle. I. Heterosis for weight, height, and maturing rate. J. Anim. Sci. 55:280-292.

Park, C. S., G. R. Fisher and C. N. Haugse. 1980. Effect of dietary protein and sunflower meal on blood serum cholesterol of dairy heifers. J. Dairy Sci. 63:1451-1461.

Park, C, S., W. Rafalowski and G. D. Marx. 1983. Effect of dietary fat supplement on lipid metabolism of Holstein heifers. J. Dairy heifers. J. Dairy Sci. 66:528-534.

Park, C. S., G. M. Erickson, Y. J. Choi and G. D. Marx. 1987. Effect of compensatory growth on regulation of growth and lactation: response of dairy heifers to a stair-step growth pattern. J. Anim. Sci. 64:1751-1758.

Park, C. S., Y. J. Choi, W. L. Keller and R. L. Harrold. 1988. Effects of compensatory growth on milk protein gene expression and mammary differentiation. FASEB J. 2:2619-2624.

Park, C. S., M. G. Baik, W. L. Keller, I. E. Berg and G. M. Erickson. 1989. Role of compensatory growth in lactation: a stair-step nutrient regimen modulates differentiation and lactation of bovine mammary gland. Growth Dev. Aging 53:159-166.

Park, C. S., M. G. Baik, W. L. Keller and W. D. Slanger. 1994. Dietary energy restriction-mediated growth and mammary development in rats. J. Anim. Sci. 72:2319-2324.

Park, C. S., R. B. Danielson, B. S. Kreft, S. H. Kim, Y. S. Moon and W. L. Keller. 1998. Nutritionally directed compensatory growth and effects on lactation potential of developing heifers. J. Dairy Sci. 81:243-249.

Philippe, C. R., S. Sonia, P. R. Aaron, S. Serge, M. Davide, C. T. Claudia, P. M. Jean, S. Josiane, R. J. Francoise and A. G. Dulloo. 2005. Redistribution of glucose from skeletal muscle to adipose tissue during catch-up fat. A link between catch-up growth and later metabolic syndrome. Diabetes 54:751-756.

Pokniak, J. A. and S. B. Cornejo. 1982. Effects of energy and protein undernutrition on productive performance and carcass, liver and digestive tract composition of broiler males. Nutr. Rep. Int. 26:319-327.

Pothoven, M. A., D. C. Beitz and J. H. Thornton. 1975. Lipogenesis and lipolysis in adipose tissue of *ad libitum* and restricted-fed beef cattle during growth. J. Anim. Sci. 40:957-962.

Rhee, Y. J., J. Y. Kim, S. K. Lee and Y. H. Song. 2005. Prediction of carcass meat quality grade by ultrasound in Hanwoo. J. Anim. Sci. Technol (Kor.). 47(6):1095-1100.

Ryan, W. J., I. H. Williams and R. J. Moir. 1993a. Compensatory growth in sheep and cattle. I. Growth pattern and feed intake. Aust. J. Agric. Res. 44:1609-1621.

Ryan, W. J., I. H. Williams and R. J. Moir. 1993b. Compensatory growth in sheep and cattle. II. Changes in body composition and tissue weights. Aust. J. Agric. Res. 44:1623-1633.

Srkar, N. K., G. A. Lodge, C. J. Williams and J. I. Elliot. 1983. The effects of undernutrition of suckled pigs on subsequent growth and body composition after nutritional rehabilitation. J. Anim. Sci. 57:34-42.

Stufflebeam, J. E., J. F. Blakely, G. B. Lasley, Thompson and D. T. Mayer. 1969. Effect of energy intake upon the levels of certain blood components in young beef heifers. J. Anim. Sci. 29:992-996.

Sul, H. S. and W. Dong. 1998. Nutritional and hormonal regulation of enzymes in fat synthesis: studies of fatty acid synthase and mitochondrial glycerol-3-phosphate acyltransferase gene transcription . Annu. Rev. Nutr. 18:331-351.

USDA. 1989. Official united states standards for grades of beef carcasses. Agric. marketing Serv. USDA. Wahington, DC.

Wilson, P. N. and D. F. Osbourn. 1960. Compensatory growth after undernutrition in mammals and birds. Biol. Rev. 35:324-363.

Yambaymaba, E. S. K., M. A. Price and G. R. Foxcroft. 1996. Hormonal status, metabolic changes, and resting matabolic rate in beef heifers undergoing compensatory growth. J. Anim. Sci. 74:57-69.