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Intensified Nursing Dramatically Accelerates Growth Performance and the Size of the Body Frame in Japanese Black and Holstein Crossbred Steers

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Abstract: Crossbred (Japanese Black male and Holstein female) steers were used to investigate the effects of feeding a large amount of high-protein milk replacer on growth performance, morphological change and myogenic regulation in skeletal muscle. Group HP (n = 7) was fed high-protein milk replacer (Crude Protein (CP): 29%, maximum intake 2.0 kg day^{-1}) and group C (n = 14) was fed a standard milk replacer (CP: 26%, 0.5 kg day^{-1}) from 56-147 days of age. Changes in Body Weight (BW), body frame measurements, plasma Insulin-like Growth Factor-1 (IGF-1) and plasma Alkaline Phosphatase (ALP) were investigated. *M. Longissimus Thoracis* (LT) samples were obtained by biopsy to investigate the myofiber type composition, diameter and the mRNA expression of Myogenic Regulatory related genes by quantitative real-time RT-PCR. A large amount of high protein milk replacer (Intensified Nursing) in group HP improved their synthesis of IGF-1 in the liver and accelerated skeletal development by maintaining high levels of plasma IGF-1 and ALP concentration, which was highly controlled by CP intake from diet. The myofiber type composition and size in the LT was not influenced by nutritional condition and aging during the nursing period. Intensified Nursing extends myofiber length rather than increasing the thickness of the myofiber, according to the increase in the size of the body frame during this phase. The expression of MyoD and IGF-1 receptors in the LT decreased at the early stage of the nursing period without the influence of nutritional condition. This suggested the change would control the level of differentiation from the myoblast to the myocyte.

Key words: Crossbred steers, intensified nursing, growth, skeletal muscle, milk, myofiber

INTRODUCTION

To produce good quality beef, it is important to maximize growth performance during the early growth stage by metabolic control. This not only produces high quality beef and reduces costs by shortening the fattening period but also decreases the amount of grain feed that is required and the amount of excreta in the stock raising environment. To increase meet production, growth performance during the early growth period must be improved. Recently, Intensified Nursing has been shown to greatly improve cattle growth (Abdelsamei *et al*., 2005). The calf obtains most of its nourishment from milk replacer and dry calf starter during the nursing period; the milk replacer that bypasses the rumen and is absorbed directly in the intestinal tract is the energy source that most influences initial growth. The energy from the milk replacer is mainly composed of fat, protein and lactose and altering the milk replacer composition accelerates growth and maturation; many studies have evaluated the effects of varying milk replacer protein and fat content (Tikofsky *et al*., 2001; Brown *et al*., 2005; Bartlett *et al*., 2006; Quigley *et al*., 2006; Velayudhan *et al*., 2008). Blome *et al*. (2003) investigated growth performance and carcass composition of Holstein bulls (2-6 weeks of age) fed milk replacer at different protein concentrations (16-26%). They demonstrated that carcass protein increased and fat decreased as the ratio of protein to energy increased in the diets of bull calves, which were fed to achieve greater average daily weight gain and body measurements. Data from these studies demonstrates the potential for altering the protein and energy content of the milk replacer to improve growth efficiency and carcass composition. Intensified Nursing by feeding high-protein milk replacer accelerates growth and maturation of young daily heifers,

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thereby improving economic performance (Tozer and Heinrichs, 2001). Intensified Nursing is a technique that can be applied to fattening cattle; however, it is important to acquire a body frame size that enables development of enough skeletal muscle during the fattening period by maximizing its capacity during the early growth phase. Abdelsamei *et al.* (2005) investigated the growth performance and carcass quality of Holstein bulls fed different amounts of milk replacer for a long time (until 200 days); this shortened the fattening period by accelerating growth and as a result the intramuscular fat content did not decrease at the time of slaughter. In addition, Kamiya *et al.* (2009) reported that the weight of Holstein bull internal secretion organs such as the liver and the spleen increased as a result of Intensified Nursing. To improve the quality and quantity of meat, crossbred steers of Japanese Black males, which have a similar meat quality to Holstein females and a higher growth capacity are produced in Japan. Thus, an experiment was designed to investigate the effects of Intensified Nursing on growth performance during the early growth stage by feeding a large amount of high-protein milk replacer. Moreover, the influence of accelerated growth during the early growing phase on mRNA expression of genes related to MRFs and morphology of the myofiber were examined.

MATERIALS AND METHODS

Experimental design: Twenty-one crossbred (Japanese Black male and Holstein female) steers (29.0±1.3 days of age and 50.0±1.3 kg BW) were acquired from local dairies in Japan. They were fed milk replacer (26% CP DM^{-1}, 24% FAT DM^{-1}, 0.5 kg day^{-1}) to standardize and accustom them to an automatic control feeder (Calf-Feeders TAP5-SA2-30-P, KFA3-MA3; Forster Technik®) and they were fed a constant amount of dry calf starter and timothy hay so that conditions by the start of the experiment were similar to those for steers held in a stall barn. Steers (56.0±1.3 days) were divided into two groups based on BW and days after birth and they were examined for about 90 days based on the general nursing period in Japan (2006/11/1-2007/1/31). Seven steers were fed a large amount (maximum intake 2.0 kg day^{-1}) of high-protein milk replacer (110% TDN DM^{-1}, 29% CP DM^{-1} and 19% FAT DM^{-1}) (group HP) and the 14 other steers were fed a control milk replacer (119% TDN DM^{-1}, 26% CP DM^{-1} and 24% FAT DM^{-1}) (group C) (maximum intake 0.5 kg day^{-1}) by automatic control feeder. The nutritional composition of milk replacer and dry calf starter is shown in Table 1. The milk replacer feeding program for group HP was changed from 0.8 kg day^{-1} at 56 days to 2.0 kg day^{-1} at 75 days, kept at 2.0 kg day^{-1} (mixed in 6-fold hot water) to 130 days and decreased to no feed during the weaning period from 131-147 days. The milk replacer feeding program for group C was kept at 0.5 kg day^{-1} (mixed in 8-fold hot water) at 56-138 days and decreased to no feed during the weaning period from 139-147 days. The dry calf starter (83% TDN DM^{-1}, 26% CP DM^{-1}) was supplied to all steers with the automatic control feeder according to the same feeding program, kept at 0.5 kg day^{-1} from 56-81 days, changed to 1.5 kg day^{-1} at 101 days and kept at 1.5 kg day^{-1} to 129 days. During the weaning period (130-147 days), the dry calf starter feeding program was changed to 4.0 kg day^{-1} (group HP) and 2.4 kg day^{-1} (group C). All steers were kept in a stall barn. The milk replacer and dry calf starter were supplied by *Zen-Raku-Ren*, the National Federation of Dairy Co-operative Associations in Japan. Timothy hay (60% TDN DM^{-1}, 10% CP DM^{-1}), water and trace mineralized salt were constantly available during the experiment.

Table 1: Composition and Intake of Dry Matter (DMI), Total Digestible Nutrients (TDNI), Crude Protein (CPI) and Fat (FATI) from the milk replacer and dry calf starter in the experiment

Items	Group HP	Group C
Composition (DM%)		
Milk replacer		
DM (%)	96.0	96.0
TDN	110.4	119.0
CP	29.2	26.0
FAT	18.8	24.0
Dry calf starter		
DM (%)	86.9	86.9
TDN	82.9	82.9
CP	25.6	25.6
FAT	4.3	4.3
Intake (kg)		
Milk replacer		
DM	139.4±1.19	41.6±0.04**
TDN	153.9±1.32	49.5±0.05**
CP	40.7±0.35	10.8±0.01**
FAT	26.2±0.22	10.0±0.01**
Dry calf starter		
DM	84.5±12.09	94.0±3.03
TDN	70.0±10.02	77.9±2.51
CP	21.6±3.10	24.1±0.77
FAT	3.6±0.52	4.04±0.13

DM: Dry Matter, TDN: Total Digestible Nutrients, CP: Crude Protein and FAT: Fat composition of milk replacer and dry calf starter. Same dry calf starter was fed to all crossbred steers. Intake data are mean±SE. **Significant difference between group HP (7 steers) and group C (14 steers) ($p<0.01$)

Feed intake and growth performance: To establish the influence of Intensified Nursing on growth performance of crossbred steers, the difference in nutritional intake from milk replacer and dry calf starter was investigated using BW and body frame measurements. The examination period was divided into four (Period 1: 56-71 days, Period 2: 72-99 days, Period 3: 100-125 days, Period 4: 126-147 days) and DMI, TDNI, CPI and FATI from milk replacer and dry calf starter during each period were calculated using records from the automatic control feeder. The BW of the steers was measured at the start

of the experiment and at the end of each period. Body frame measurements (wither height, hip height, body length, heart girth and abdominal circumference) were measured at the start, 84, 113 days and end of the experiment and the thurl width was measured at the start and end of the experiment.

Measurements of plasma IGF-1 concentration: To establish the influence of Intensified Nursing on internal growth factor secretion of crossbred steers, the change in concentration of Insulin-like Growth Factor-1 (IGF-1) in the plasma was investigated. Blood samples were drawn from the jugular vein into tubes containing heparin sodium at four time points (56, 72, 99, 126 and 148 days). Plasma was separated by centrifugation (3,000 rpm for 20 min) at a temperature of 4°C and stored at -40°C until plasma IGF-1 concentrations were measured. After removal of binding proteins, concentrations of IGF-1 in the plasma were determined by radioimmunoassay (kit IGF-1 IRMA Daiichi) at SRL, Inc. Tokyo.

Measurements of plasma Alkaline Phosphatase (ALP) concentration: Plasma concentration of ALP was measured as a submarker of bone formation because it was easy to measure it (Charles *et al*., 1985). Using same blood plasma at 4 time points, ALP concentration was measured by using the Spot ChemII Auto Analysis System (SP-4410, Arkray).

Calculation of myofiber type composition and myofiber size: After giving anesthesia to the experimental animals, *M. Longissimus Thoracis* (LT) samples were obtain from steers aged 41-148 days by biopsy for investigation of myofiber type (type I: slow-twitch oxidative, type IIA: fast-twitch oxidative glycolytic, type IIB: fast-twitch glycolytic) composition and the associated sizes of the three myofiber types. They were rapidly frozen in liquid nitrogen and stored at -80°C until cutting into serial frozen sections. The serial frozen sections (8 μm thick) were cut transversely (CM 1850, Leica) and stained using two histochemical reactions to observe the distribution of the myofiber types. The myosin Adenosine Triphosphatase (ATPase) activities were detected afteracid (pH 4.3) or alkaline (pH 9.4) preincubation (Brooke and Kaiser, 1969). Reduced Nicotinamide Adenine Dinucleotide Dehydrogenase (NADH-DH) activity (Okamoto *et al*., 1976) was also measured. On two series of photographs (x250), the myofibers were divided into types I, IIA and IIB according to the nomenclature of Brooke and Kaiser (1970). For a total of 300 myofibers on each photograph, the diameter of the three myofiber types was calculated at the largest width perpendicular to the long axis (Brooke, 1970).

RNA isolation and quantitative real-time reverse transcription PCR: Using the LT samples by biopsy at 41 and 148 days, mRNA expression of Myogenic Regulatory Factors (MRFs: Myogenin, Myf5, MyoD, Myostatin, MRF4, IGF-1 and IGF-1recepter) was investigated. The samples were rapidly frozen in liquid nitrogen and stored at -80°C until total RNA isolation. Total RNA was isolated from smashed LT muscle (Micro Smash™ MS-100, TOMY) using ISOGEN reagent (Nippon Gene) according to the manufacturer's instructions. Purity and quantity of RNA were determined using a spectrophotometer (GeneQuant RNA/DNACalculator, GE Healthcare) and accepted samples had a ratio of optical measurements at 260 and 280 nm (OD260 nm/OD280 nm) of >1.8. Single stranded cDNA were reverse transcribed from total RNA 1 μg using the First Strand cDNA Synthesis kit (ReverTra Ace-α-®, TOYOBO) according to the manufacturer's instructions. Two microliters of cDNA solution was used in each reaction, along with 5 μL of SYBR Green Dye (SYBR® Green Realtime PCR Master Mix, TOYOBO) and 10 μM of forward and reverse primer. PCR conditions were 95°C for 1 min, 95°C for 15 sec, 59°C for 15 sec and 72°C for 30 sec. Real-time PCR reactions were performed in a Line Gene Real-Time PCR System (BioFlux). Relative mRNA abundances of Myogenin, Myf 5, MyoD, Myostatin, MRF4, IGF-1 and IGF-1 receptor were expressed as the fold difference in the expression of these target genes relative to the expression of the endogenous reference gene Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH). Primer sequences used for each gene are shown in Table 2. These primers were designed using Primer Software DNASIS® Pro (Hitachi Software Engineering).

Table 2: Sequence of primer pairs used for determining the relative abundance of mRNA for myogenic regulatory factors in the skeletal muscle of crossbred steers

Gene	Forward	Reverse	Amplification size (bp)
Myogenin	5'-CAGAGGCTGCCCAAAGTGGAG-3'	5'-CCAAACTCCAGTGCGCTGC-3'	200
Myostatin	5'-GGAAGACGATGACTACCACGC-3'	5'-TGCACAAACACTGTCGCAGG-3'	198
MYF5	5'-GCTCTGATGG CATGCCTGA-3'	5'-GCTCTGAGTTGGTGATCCG-3'	168
MyoD	5'-GAACACTACAGCGGCGACTC-3'	5'-TAGTAAGTGCGGTCGTAGCAG-3'	122
MRF4	5'-CATCGTGGACAGCATTTCCTCG-3'	5'-TCATCCGAGCGTGCACAGCA-3'	241
IGF-1	5'-CATCACATCCTCCTCGCATCTC-3'	5'-GTACATCTCCAGCCTCCTCAG-3'	250
IGF-1recptor	5'-GTGACGTCCTGCACTTCACC-3'	5'-CCATGTTCCAGCTGTTGGAGC-3'	186
GAPDH	5'-GCCGTAACTTCTGTGCTGTGCC-3'	5'-TCTCTGCCTTGACTGTGCCG-3'	226

GAPDH (glyceraldehyde-3-phosphate dehydrogenase) as internal control

Statistical analysis: Data were expressed as mean±SE and statistically analyzed using Student's t-test. Significance was declared at $p<0.05$ unless otherwise noted. Investigations were conducted in accordance with the National Research Council Publication, Guide for Care and Use of Laboratory Animals. The animal Experiment Committee of Kyushu University investigated the research plan and granted permission for the animal experiments (document number A19-128-1).

RESULTS AND DISCUSSION

Nutrient intake of milk replacer and dry calf starter and growth performance: The total intake of Dry Matter (DMI) during the experiment was calculated from the Total Digestible Nutrients (TDNI), Crude Protein (CPI) and Fat (FATI) from milk replacer and dry calf starter in each group as shown in Table 1. The change in DMI from milk replacer (Fig. 1a) and dry calf starter (Fig. 1b) and total CPI from milk replacer and dry calf starter (Fig. 1c) at four periods are shown in Fig. 1. All steers were fed milk replacer according to the automatic control feeder program (by design) and total DMI of milk replacer was higher in group HP than in group C ($p<0.01$) (Table 1 and Fig. 1a). Group C consumed dry calf starter according to the feeding program but intake in group HP was lower than that by the feeding program. In group HP, dry calf starter intake increased with decreasing milk replacer intake during the weaning period 4 (126-147 days) and reached about 3.0 kg day^{-1} at the end of the experiment.

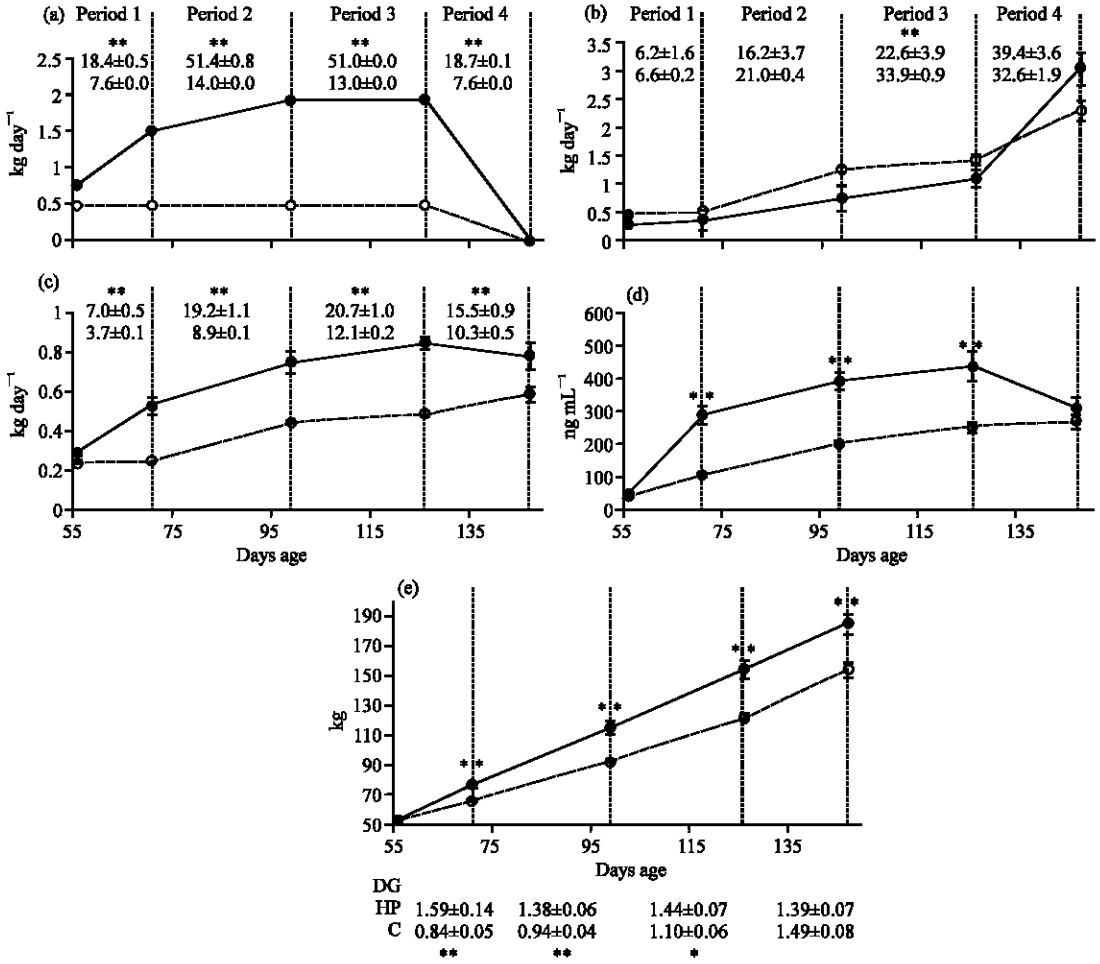

Fig. 1: Change in the dry matter intake of milk replacer (a), dry calf starter (b), total crude protein intake of experimental diets (c) concentrations of plasma insulin-like growth factor-1 (d) and bodyweight (e) of group HP (●) and group C (○). The examination period was divided into four (Period 1: 56-71 days of age (d), Period 2: 72-99 days, Period 3: 100-125 days and Period 4: 126-147 days). The bar extending from the data points indicates the SE. *,**Significant difference between group HP and group C ($p<0.05$, $p<0.01$, respectively)

DMI from dry calf starter was significantly higher in group C than in group HP at period 3 ($p<0.01$) but the nutrient intake from dry calf starter was not significantly different between groups for all of the experimental period (Fig. 1b). In group HP, total CPI from milk replacer and dry calf starter increased with decreased milk replacer intake at period 4 but increased at the other periods and was significantly higher than that in group C at all periods ($p<0.01$) (Fig. 1c).

The change in BW at each period is shown in Fig. 1e. BW (77.1±3.8 kg) was higher in group HP than in group C (65.8±1.5 kg) at 72 days ($p<0.01$) and the Relative Daily Gain (RDG) was higher in group HP than in group C until period 3 ($p<0.05$). The final BW (185.3±7.2 kg) was higher in group HP than in group C (154.5±4.8 kg) (Fig. 1e). Changes in body frame measurements (wither height, hip height, body length, heart girth, abdominal circumference and thurl width) are shown in Fig. 2. Daily growth (cm day^{-1}) of wither height, hip height, body length and heart girth was significantly higher in group HP than in group C initially (from 50-84 days) ($p<0.05$) and the abdominal circumference was larger in group HP than in group C during the middle term (from 84-113 days) ($p<0.01$). Daily growth (cm day^{-1}) of thurl width was significantly higher in group HP than in group C during the experiment ($p<0.01$) and the final wither height, body length, heart girth and thurl width were significantly larger in group HP than in group C ($p<0.05$).

Change in plasma IGF-1 concentrations: The change in plasma IGF-1 concentrations is shown in Fig. 1d. IGF-1 concentration was higher in group HP (289±25.7 ng mL^{-1}) than in group C (107±6.1 ng mL^{-1}) at 72 days ($p<0.01$) and the concentration increased dramatically in group HP compared with that in group C ($p<0.01$) until 126 days when milk replacer intake in group HP began to decrease. However, there was no difference in plasma IGF-1 concentration between groups at the end of the experiment.

Change of plasma Alkaline Phosphatase (ALP) concentration: ALP concentration in both groups showed peaks at 99 days of age and then gradually decreased to the age of 146 days. ALP concentration in group HP, however, was significantly higher than group C at 72 days age ($p<0.05$). Moreover, it also showed a higher tendency in group HP than that in group C at 99 days of age ($p<0.10$) (Fig. 3).

Myofiber type composition and myofiber size: Percentage distributions of myofiber types (type I, type IIA and type

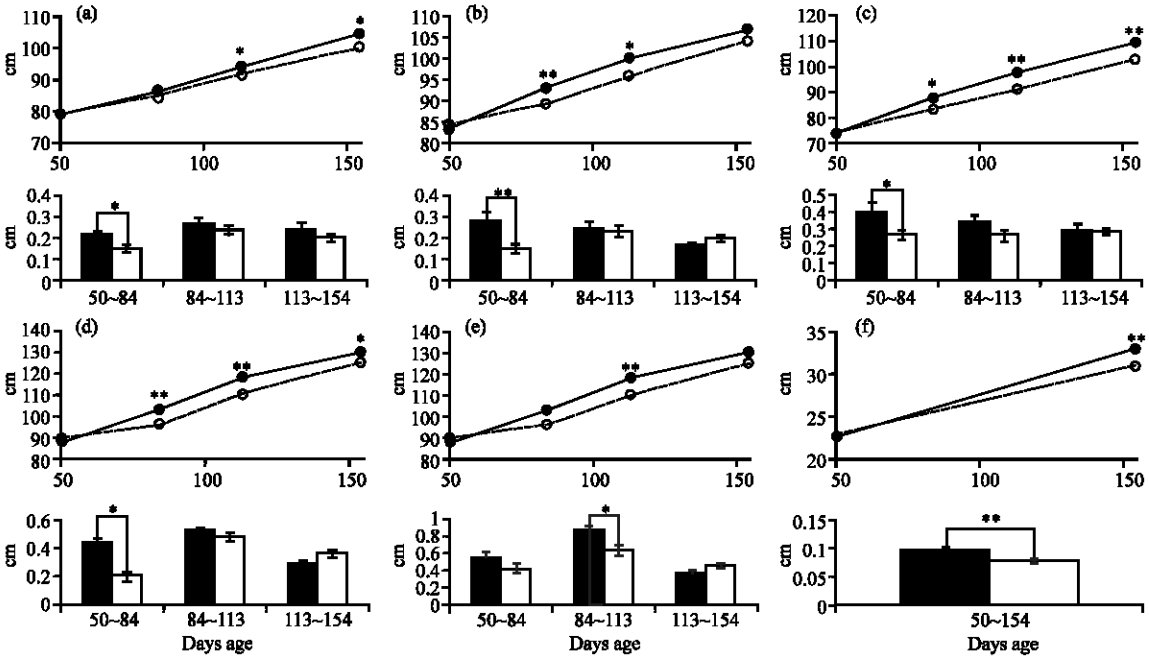

Fig. 2: Changes in the distributions of body frame measurements (wither height (a), hip height (b), body length (c), heart girth (d), abdominal circumference (e) and thurl width (f)) of group HP (●) and group C (○) and growth rate at 3 periods (50-84, 84-113 and 113-154 days) of group HP (■) and group C (□). Data are mean±SE. *,**Significant difference between group HP and group C ($p<0.05$, $p<0.01$, respectively)

Table 3: Percentage distributions of myofiber types and diameters of myofiber in each type at the start (41 days) and the end (148 days) of the experiment

	Percentage distribution						Diameter of myofibers (μm)					
	Type I (day)		Type IIA (day)		Type IIB (day)		Type I (day)		Type IIA (day)		Type IIB (day)	
Groups	41	148	41	148	41	148	41	148	41	148	41	148
HP	25.1±1.7	23.1±2.7	27.3±2.8	25.1±3.9	47.6±3.1	51.7±4.0	23.7±1.3	30.0±2.3	27.6±2.6A	40.7±2.1B	38.4±3.0	51.4±5.9
C	23.5±1.3	22.3±1.8	24.6±1.5	27.3±2.5	51.8±1.4	50.4±1.9	22.8±1.1A	27.8±1.1B	30.0±2.3A	37.5±1.1B	37.7±2.5a	47.9±2.6b

Data are mean±SE. a-b: $p<0.05$, A-B: $p<0.01$ between 41 and 148 days of age for each group

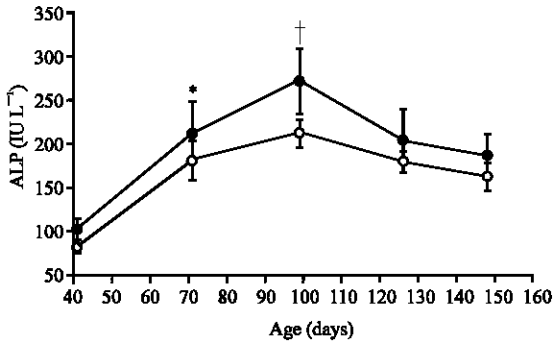

Fig. 3: Change in Alkaline Phosphatase (ALP) concentration of plasma for group HP (●) and group C (○). The bar extending from the data points indicates the SE. *$p<0.05$. †$p<0.10$

IIB) and diameter of myofibers in LT samples obtained from steers at 41 and 148 days by biopsy are shown in Table 3. There was no difference in myofiber type composition between groups at 148 days and there was no change in myofiber type composition in each group between 41 and 148 days. However, differences were seen in the pattern of change in the diameter of myofibers between groups. The diameter of all myofiber types in group C increased ($p<0.05$) during the experiment, though only the diameter of type IIA myofiber increased in group HP ($p<0.01$).

mRNA expressions of the target genes: Fold differences in expression of Myogenic Regulatory Factors (MRFs: Myogenin, Myf5, MyoD, Myostatin, MRF4, IGF-1 and IGF-1recepter) in LT samples obtained from steers at 41 and 148 days by biopsy, relative to the endogeneous reference gene, GAPDH are shown in Fig. 4. The relative expression of Myogenin was significantly lower in group HP than in group C ($p<0.05$) though there was no difference in the relative expression of the other genes between groups at 148 days. The results on the pattern of change in the relative expression of MRFs showed a significant decrease in MyoD and IGF-1 receptor in each group between the start and end of the experiment ($p<0.05$). Therefore, there was a significant decrease in Myogenin and Myostatin and an increase in MRF4 in group HP between the start and end of the experiment ($p<0.05$).

Relationship between milk replacer and dry calf starter intake: Calf muscle bulk increases by feeding a milk replacer that has a high concentration of Crude Protein (CP). Bartlett *et al.* (2006) investigated the effect of protein content on growth performance and body composition of 59 Holstein bulls fed (1.25 or 1.75%/BW) the same energy level of milk replacer but with a CP content at four levels (14-26%). They reported that fat accumulation in the body decreased and protein accumulation and DG increased as CP content in the milk replacer increased and protein accumulation decreased and fat accumulation increased as milk replacer intake increased. Tikofsky *et al.* (2001) reported that growth performance of bull calves fed high fat milk replacer (31%) did not differ and that the calves had more fat and less protein accumulation in the body compared with one other calf that was fed a different milk replacer (15 and 22% FAT). Hill *et al.* (2008) investigated growth performance and body composition of 24 Holstein bulls fed milk replacer in groups with different nutrition levels (LPLF: 20% CP, 21% FAT, DMI 441 g day^{-1}, HPLF: 28% CP, 20% FAT, DMI 951 g day^{-1}, HPHF: 27% CP, 28% FAT, DMI 951 g day^{-1} and HPHF(+): 27% CP, 28% FAT, DMI 1,431 g day^{-1}). They reported a similar result to Tikofsky *et al.* (2001) fat accumulation in the body of group HPLF decreased and DG in group HPLF did not differ from that in group HPHF. Additionally, dry calf starter intake in group HPHF (+) was lower than that in group HPHF. These studies suggest that an increase in the intake of milk replacer and simultaneously feeding a high-fat replacer reduce the dry calf starter intake and accelerate body fat accumulation.

In this study, high growth performance of the crossbreed cattle before weaning was exhibited in group HP, where the CP and FAT compositions in the milk replacer were 3% higher and 5% lower, respectively, than those in group C. In group HP, the CPI and FATI from milk replacer increased more than those in group C by about 30 and 16 kg, respectively, during an artificially controlled program using an automatic control feeder. DMI from dry calf starter was lower in group HP than in group C at period 3. This suggests that feeding a large amount of low fat milk replacer maximized fat intake and resulted in a decreasing intake of dry calf starter. Furthermore, the intake of dry calf starter and its digestion

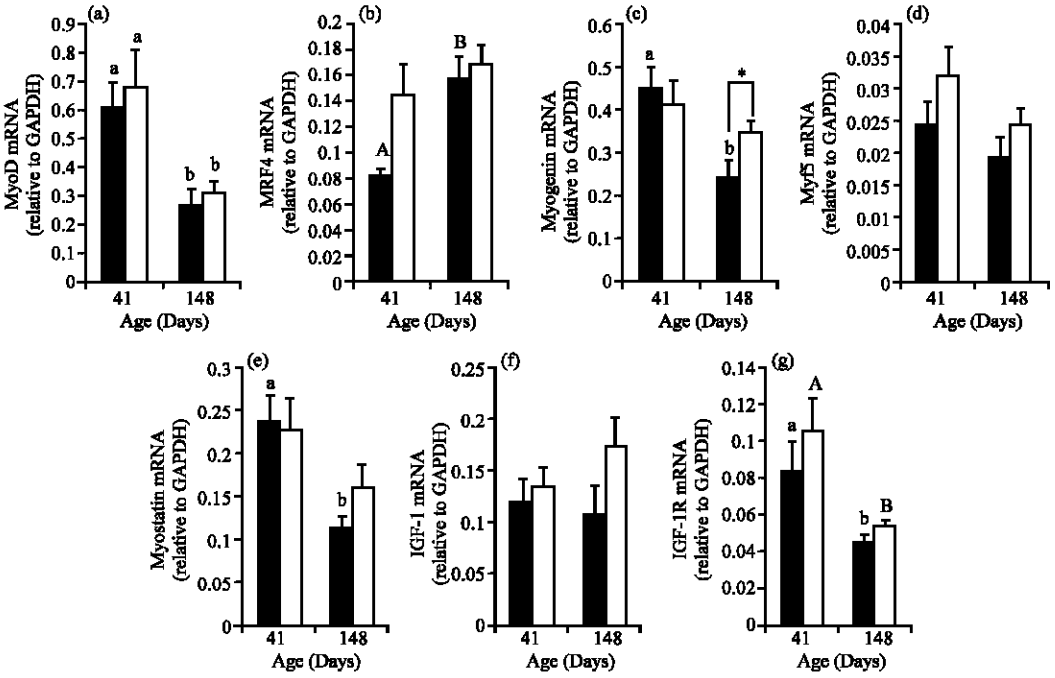

Fig. 4: Fold difference in the expression of mRNA of MyoD (a), MRF4 (b), Myogenin (c), Myf5 (d), Myostatin (e), insulin-like growth factor (IGF)-1 (f) and IGF-1 receptor and (g) in the skeletal muscle (*M. Longissimus thoracis*) relative to the endogeneous reference gene glyceraldehyde-3-phosphate dehydrogenase(GADPH). Group HP: (■), group C: (□). The bar extending from the data points indicates the SE. Data are mean±SE. a-b, A-B Significant difference between 41 and 148 days of age in the same group ($p<0.05$, $p<0.01$, respectively). *Significant difference between group HP and group C ($p<0.05$)

and absorption are closely related to the developmental stage of the digestive organ, including the rumen, during the nursing period. In general, it is thought that the rumen develops rapidly by the stimulation of dried diet intake at 4-8 weeks of age and its development is influenced by the existence of bacillus, the aquatic environment and movement in the rumen and nourishment absorption by mucosal epithelia. Kristensen *et al.* (2007) investigated the effect of milk replacer intake on dry calf starter intake, ruminal milieu and three fore-stomachs. Dry calf starter intake was inhibited by excessive milk replacer intake and the rumen and omasum weight of a calf fed a high amount of dry calf starter were heavier than those of a calf fed a small amount. However, the difference in dry calf starter intake did not influence the growth of the villus in the caudoventral blind and the ventral sac in front of the rumen.

In this study, there was a difference in dry calf starter intake and a change in patterns between groups. DMI of dry calf starter in group C was higher than that in group HP at period 3, though there was no difference up to period 2. We suggest that the capacity (weight) of the rumen developed earlier in group C than in group HP.

There was no difference in dry calf starter intake between groups but intake in group HP increased with a decrease in milk replacer intake during period 4, which was at the time of the weaning process. This suggests that in group HP the capacity (weight) of the rumen of steers increased during this period. The effect of hay intake on the decomposition speed in the rumen and absorption by the mucosal epithelia was less than that of dry calf starter intake (Anderson *et al.*, 1987). However, the details of the influence of hay intake on the rumen and omasum should be examined.

Effect of milk replacer and dry calf starter intake on plasma IGF-1 concentration and growth performance: The Insulin-like Growth Factors (IGFs) are polypeptides with a high sequence similarity to insulin. The IGF-1 is mainly secreted by the liver as a result of stimulation by Growth Hormone (GH) and it strongly influences most cells of animals, especially the muscle and bone (Cohen *et al.*, 1991). Barttlett *et al.* (2006) reported that total CPI had a higher positive correlation with plasma IGF-1 concentration than did the metabolizable energy intake during the nursing period. The study supports this

result; the pattern of change in plasma IGF-1 concentration (Fig. 1d) was remarkably similar to that in total CPI from milk replacer and dry calf starter (Fig. 1c). In general, the circulation IGF-1 in the plasma is mostly bonded to some IGF Binding Proteins (IGFBPs) and the function of IGF-1 is adjusted by IGFBPs (Hossner *et al*., 1997). The IGFBP-2 acts on cell growth and assimilation during the early growth period of the calf, while reacting to protein nourishment under a low energy intake situation. In addition, the concentration of plasma IGF-1 and the composition ratio of IGFBP-2 to IGFBP-3 are adjusted according to growth (McGuire *et al*., 1992; Lee *et al*., 2000). It is generally thought that smooth absorption of the milk replacer protein in the duodenum influences plasma IGF-1 concentration with changes in the synthesis of IGFBP-2 and other IGFBPs during the nursing period. However, in this study, the concentration of plasma IGF-1 was quadratically correlated with total CPI (Fig. 5). The regression equation for the predicted plasma IGF-1 concentration (y) from the total daily CPI (x) data is $y = 1507.5092x\text{-}1005.7685x^2\text{-}161.4552$ ($R^2 = 0.6209$). We suggest that excessive CPI did not necessarily promote efficient IGF-1 synthesis in the liver during the nursing period. This might have been responsible for the remarkable change in the balance of intake between milk replacer and dry calf starter during the weaning period (period 4) because there was a difference in the digestibility and absorption efficiency of protein in the milk replacer (liquid) and dry calf starter (solid). The intake of dry calf starter increased rapidly with the decrease in milk replacer intake during the weaning period (period 4). In group HP, a large change in the total CPI changed was observed (0.6 kg day^{-1} or more) (Fig. 1c). However, in this period, functioning of the reticulo rumen and its ability to digest and absorb the grain diet seemed not yet fully developed. We suggest that the amount of protein that was smoothly absorbed in the duodenum decreased for the following reasons: there was a large decrease in CPI from the milk replacer with a high efficiency of utilization, the dry calf starter was trapped by the undeveloped reticulo rumen and it took a long time to digest the solid dry calf starter in the abomasums, which did not have sufficient digestive function. As a result, there was no difference in the plasma IGF-1 concentration between the two groups, though DMI from the dry calf starter in group HP (3.03±0.29 kg) was significantly higher than that in group C (2.29±0.18 kg) ($p<0.01$) at 147 days of age when weaning was complete. Therefore, for efficient synthesis of IGF-1 and growth performance, it is important to not only maintain high CPI but also to shift to an absorbable protein source. This takes into consideration the development of the digestive organ in the abomasum and

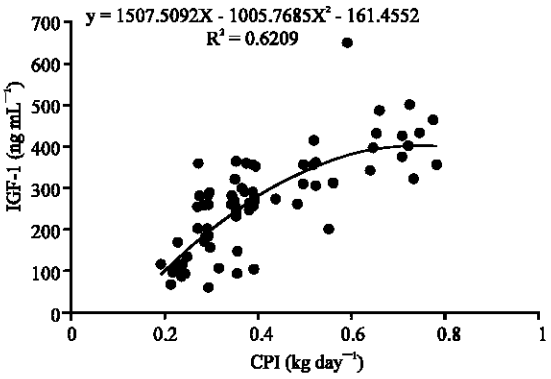

Fig. 5: Relationship between total daily CP intake (kg day^{-1}) and concentration of plasma insulin-like growth factor-1 (IGF-1 ng mL^{-1}) plotted for individual crossbred steers at 56, 71, 99, 126 and 147 day

reticulo-rumen during weaning. In group HP, BW and size of the body frame increased as a result of Intensified Nursing, mainly because it promoted IGF-1 synthesis in the liver according to the increase in CPI from the milk replacer. In particular, DG and daily growth of most the body frame measurements were very high until 75 days; this suggests that the milk replacer was used more efficiently during the early growth phase. In group HP, except for abdominal circumference, there was a rapid increase in the daily body frame measurements at the initial stage (50-84 days) but there was no difference in these measurements between groups during the later stages (84-154 days). However, RDG was higher in group HP than in group C until 125 days. Hammond (1932) reported that the growth of the nerves and bone took precedence over that of the muscle and fat, as derived from standard curves of the growth of several organs in livestock.

We suggest that growth of bone was given priority as a result of Intensified Nursing during the initial stage and BW increased with development of the skeletal muscles and fat accumulation during the later stages. In group HP, only the daily growth of the abdominal circumference increased at 84-113 days. This suggests that curd formed in the abomasum as a result of a large amount of milk replacer, which increased the volume of the rumen, rather than the enlargement of the rumen by dry calf starter intake as was observed in group C. This supports the results from the daily growth of the abdominal circumference in group HP, which showed a rapid decrease at 113-154 days.

ALP synthesized in the bone was produced at a higher level during growth. From the results of the plasma ALP concentration, there were significant differences at 72 ($p<0.05$) and 99 ($p<0.10$) days (Fig. 3): group HP

showed a higher level of ALP than that of group C at both ages. This suggests that body frame development of calves in group HP was more rapid than that in group C during this phase.

Change in myofiber type composition and size according to skeleton formation: In general, the myofiber type composition in the skeletal muscle of cattle differs according to cattle type, age, sex, muscular parts and the feeding environment (Gotoh, 2003). Greenwood *et al.* (2009) investigated the composition and sectional area ratio of myofiber type in LT muscle of cattle reared in restricted energy conditions that decreased body weight by 10% at 115 days. They reported that the sectional area ratio of type I myofiber in the LT decreased and there was no difference in myofiber type composition compared with that of cattle reared with sufficient energy (DG during rearing period: 0.2-0.6 kg day^{-1}). This supports the results; the diameters of myofiber type I and IIA in group C increased with growth, though there was no difference in the myofiber type composition and diameter between groups at the end of the experiment. In contrast, in group HP, only in myofiber type IIA was a similar tendency observed. We suggest that bone growth was given priority over that of the muscle, especially during the nursing period. Therefore, the skeletal muscles might have repeated differentiation and growth along the direction of the myofibers and according to the growth of the bone as a result of Intensified Nursing. In particular, body length growth was very high in this study and we believe that the LT was expanded and adjusted to the size of the frame, rather than a thickening of the sectional area. In addition, thurl width in group HP was significantly longer than that in group C at the end of the experiment ($p<0.01$); therefore, the width of LT muscle bonded to the os coxae might have been enhanced. Enhancing skeletal muscles with bone growth is thought to be an important technique that will be used in the future for accelerating meat production. Gotoh *et al.* (1999) reported that the compositions of type I, IIA and IIB myofiber were 23.8, 24.1 and 52.1%, respectively, in the LT muscle of Japanese Black steers at 11 months and type I myofiber seemed to shift to type IIA during fattening. We suggest that the change in myofiber type composition in LT muscle with aging was influenced by the condition during the fattening period, because a change in myofiber type composition resulting from nutritional condition and growth was not observed during the nursing period in this study.

MRFs trends during the early growing phase: To improve beef production, it is important to accelerate the growth of the skeletal muscles. In the differentiation of myocytes that construct muscle, Myogenic regulatory factors (MRFs: MyoD, Myf5, myogenin and MRF4) are the master genes, (i.e., MyoD family), which have the ability to induce differentiation (Wright *et al.*, 1989; Hinterberger *et al.*, 1991; Weintraub, 1993; Megeney and Rudicki, 1995). In this study, the difference in the nutritional level during the nursing period, which had a marked effect on mRNA expression of the MyoDs genes, was investigated as a way of improving meat productivity. MRFs are basic-helix-loop-helix (bHLH) transcription factors that regulate myogenesis. Part of the function of the MyoD family as master genes is thought to be activation of transcription in specific genes of the skeletal muscle. These proteins contain a conserved basic DNA binding domain that binds the E box DNA motif (Murre *et al.*, 1989). They form dimers with other HLH containing proteins through an HLH-HLH interaction. Some evidence suggests that MRFs may also play a more extended role in the maintenance of mature skeletal muscle fiber phenotypes. When cells with the genealogy of skeletal muscle appeared in the sarcomere, MyoD and Myf5 expression changed the cells into myoblasts. The expression of myogenin and MRF4 genes is well known to be differentiated myotube markers. Myogenin functions to differentiate the myoblast into the myotube and maintain the activity of the myotube and MRF4 plays a role in the maturity and final differentiation control of the myotube. In addition, IGF-1 is the main growth factor in skeletal muscles.

In this study, expression of MyoD and IGF-1 receptor decreased with growth in both groups. We suggest that the levels of myofiber differentiation and formation of IGF-1 receptor in the skeletal muscle decreased with rapid growth, without the influence of nutritional condition during the early growing phase. There was no difference in the expression of Myf5 and IGF-1 between group HP and group C and the gene expressions did not change during the early growth period. This suggests that myofiber differentiation was mainly controlled by the MyoD and the IGF-1 activity in the skeletal muscle maintained the balance by adjusting the intake of circulating IGF-1, instead of taking in paracrine IGF-1. Shibata *et al.* (2006) investigated the change in mRNA expression of Myostatin as an inhibitor of proliferation of myoblasts in the semitendinosus muscle during the rearing period (2-10 months) of Japanese Black Cattle. Myostatin expression was highest at 2 months of age and then decreased gradually until 10 months when it was half of that at 2 months. In group HP, at the end of experiment (148 days) Myostatin expression decreased to about half of that at the start (41 days). Comparing the results of Shibata *et al.* (2006) with the results from this study, we suggest that in group HP the decrease in Myostatin expression was accelerated with growth. In general, the order of activation is MyoD, Myf5, Myogenin and MRF4

and it is thought that MyoD and Myf5 adjust myotube formation during the first stage and that Myogenin and MRF4 adjust myotube formation during the later stages (Muroya *et al*., 2002). In this study, in group HP, Myogenin expression was significantly lower than that in group C at the end of the study ($p<0.05$). We suggest that the influence of Intensified Nursing was greatest at the later stage of the differentiation process from the myoblast to the myotube. At the start of the study, the mean expression of MRF4 differed between group HP and group C, though there was no statistically significant difference in the expression of MRF4 between groups because of the large standard error in group C. When the data from two of the steers in group C, which had an MRF4 expression of 0.3 or more (relative to GAPDH) at the start of the study, were excluded, the mean±SE was adjusted to 0.113±0.016. In this case, in group C, there was no significant difference in the expression of MRF4 between the start and end of the study ($p = 0.068$) and the tendency to increase its expression with growth was reduced. This suggests that in group HP there might have been an exponential increase in the expression of MRF4 as a result of Intensified Nursing. Intensified Nursing rapidly caused the expression of Myogenin to decrease control of myogenic differentiation. Moreover, in group HP, myotube formation might have progressed rapidly by a decrease in the expression of Myostatin and an increase in the expression of MRF4.

CONCLUSION

Crossbreed (Japanese Black male and Holstein female) steers fed a large amount of high-protein milk replacer (Intensified Nursing) improved their synthesis of IGF-1 in the liver and accelerated skeletal development by maintaining high levels of plasma IGF-1 concentration. This trend was especially marked during the early phase of nursing. Morphological change in the LT was not influenced by nutritional condition and aging during the nursing period. Intensified Nursing extends myofiber length rather than increasing the thickness of the myofiber, according to the increase in the size of the body frame during this phase. The expression of MyoD and IGF-1 receptors in the LT decreased at the early stage of the nursing period without the influence of nutritional condition and this change controlled the level of differentiation from the myoblast to the myocyte. However, myotube formation might have progressed rapidly because of a decrease in the expression of Myostatin and an increase in the expression of MRF4 might have been strongly accelerated as a result of Intensified Nursing.

REFERENCES

Abdelsamei, A.H., D.G. Fox, L.O. Tedeschi, M.L. Thonney, D.J. Ketchien and J.R. Stouffer, 2005. The effect of milk intake on forage intake and growth of nursing calves. J. Anim. Sci., 83: 940-947.

Anderson, K.L., T.G. Nagaraja and J.L. Morrill, 1987. Ruminal metabolic development in calves weaned conventionally or early. J. Dairy Sci., 70: 1000-1005.

Bartlett, K.S., F.K. McKeith, M.J. Vandehaar, G.E. Dahl and J.K. Drackley, 2006. Growth and body composition of dairy calves fed milk replacers containing different amounts of the protein at two feeding rates. J. Anim. Sci., 84: 1454-1467.

Blome, R.M., J.K. Drackley, F.K. McKeith, M.F. Hutjens and G.C. McCoy, 2003. Growth, nutrient utilization and body composition of dairy calves fed milk replaces containing different amounts of protein. J. Anim. Sci., 81: 1641-1655.

Brooke, M.H. and K.K. Kaiser, 1969. Some comments on the histochemical characterization of muscle adenosine triphosphatase. J. Histochem. Cytochem., 17: 431-432.

Brooke, M.H. and K.K. Kaiser, 1970. Muscle fiber types: How many and what kind? Arch. Neurol., 23: 369-379.

Brooke, M.H., 1970. Some Comments on Neural Influence on the Two Histochemical Types of Muscle Fibers. In: The Physiology and Biochemistry of Muscle as a Food, Briskey, E.J., R.G. Cassens, B.B. Marsh (Eds.). Vol. 2, University of Wisconsin Press, Madison, WI., USA., pp: 131-153.

Brown, E.G., M.J. Vandehaar, K.M. Daniels, J.S. Liesman, L.T. Chapin, D.H. Keisler and M.S.W. Nielsen, 2005. Effect of increasing energy and protein intake on body growth and carcass composition of heifer calves. J. Dairy Sci., 88: 585-594.

Charles, P., J.W. Poser, L. Mosekilde and F.T. Jensen, 1985. Estimation of bone turnover evaluated by 47Ca-kinetics efficiency of serum bone gamma-carboxyglutamic acid-containing protein, serum alkaline phosphatase and urinary hydroxyproline excretion. J. Clin. Invest., 76: 2254-2258.

Cohen, P., D.M. Peehl, G. Lamson and R.G. Rosenfeld, 1991. Insulin-like growth factors (IGFs), IGF receptors and IGF-binding proteins in primary cultures of prostate epithelial. J. Clin. Endocrinol. Metab., 73: 401-407.

Gotoh, T., 2003. Histochemical properties of skeletal muscles in Japanese cattle and their meat production ability. Anim. Sci. J., 74: 339-354.

Gotoh, T., S. Imamura, H. Iwamoto and Y. Ono, 1999. Comparative study on the myofiber type composition and the fat deposit in *M. longissimus thoracis* between young heifer groups of Japanese Black fed freely on concentrate and roughage. Proceedings of the 45th International Congress of Meat Science and Technology, Aug. 1-6, Yokohama, Japan, pp: 278-297.

Greenwood, P.L., N.W. Tomkins, R.A. Hunter, P.G. Allingham, S. Harden and G.S. Harper, 2009. Bovine myofiber characteristics are influenced by post-weaning nutrition. J. Anim. Sci., 87: 3114-3123.

Hammond, M.A., 1932. Growth and the Development of Mutton Qualities in the Sheep. Oliver and Boyd, Edinburgh, London.

Hill, S.R., K.F. Knowlton, K.M. Daniels, R.E. James, R.E. Pearson, A.V. Capuco and R.M. Akers, 2008. Effects of milk replacer composition on growth, body composition and nutrient excretion in preweaned *Holstein heifers*. J. Dairy Sci., 91: 3145-3155.

Hinterberger, T.J., D.A. Sassoon, S.J. Rhodes and S.F. Konieczny, 1991. Expression of the muscle regulatory factor MRF4 during somite and skeletal myofiber development. Dev. Biol., 147: 144-156.

Hossner, K.L., R.H. McCusker and M.V. Dodson, 1997. Insulin-like growth factors and their binding proteins in domestic animals. Anim. Sci., 64: 1-15.

Kamiya, M., M. Matsuzaki, H. Orito, Y. Kamiya, Y. Nakamura and E. Tsuneishi, 2009. Effects of feeding level of milk replacer on bodygrowth, plasma metabolite and insulin concentrations and visceral organ growth of suckling calves. Anim. Sci. J., 80: 662-668.

Kristensen, N.B., J. Sehestd, S.K. Jensen and M. Vestergaard, 2007. Effect of milk allowance on concentrate intake, ruminal environment and ruminal development in milk-fed *Holstein calves*. J. Dairy Sci., 90: 4346-4355.

Lee, H., R.S.A. Vega, L.T. Phung, N. Matsunaga, H. Kuwayama and H. Hidari, 2000. Changes in plasma insulin-like growth factor (IGF)-1, IGF binding protein (IGFBP)-2 and IGFBP-3 during fasting in *Holstein* adult steers and calves. Anim. Sci. J., 71: 178-188.

McGuire, M.A., J.L. Vicini, D.E. Bauman and J.J. Veenhuizen, 1992. Insulin-like growth factors and binding proteins in ruminants and their nutritional regulation. J. Anim. Sci., 70: 2901-2910.

Megeney, L.A. and M.A. Rudicki, 1995. Determination versus differentiation and the MyoD family of transcription factors. Biochem. Cell Biol., 73: 723-732.

Muroya, S., I. Nakajima and K. Chikuni, 2002. Sequential expression of myogenic regulatory factors in bovine skeletal muscle and the satellite cell culture. Anim. Sci. J., 73: 375-381.

Murre, C., P.S. McCaw and D. Baltimore, 1989. A new DNA binding and dimerization motif in immunoglobulin enhancer binding, daughterless, MyoD and myc proteins. Cell, 56: 777-783.

Okamoto, K., U. Ueda, R. Maeda, A. Mizutani and T. Sugiyama, 1976. Microscopic Histochemistry. Vol. 1, Igaku-Shoin Ltd., Tokyo.

Quigley, J.D., T.A. Wolfe and T.H. Elsasser, 2006. Effects of additional milk replacer feeding on calf health, growth and selected blood metabolites in calves. J. Dairy Sci., 89: 207-216.

Shibata, M., K. Matsumoto, K. Aikawa, T. Muramoto, S. Fujimura and M. Kadowaki, 2006. Gene expression of myostatin during development and regeneration of skeletal muscle in Japanese Black cattle. J. Anim. Sci., 84: 2983-2989.

Tikofsky, J.N., M.E. van Amburgh and D.A. Ross, 2001. Effect of varying carbohydrate and fat content of milk replacer on body composition of Holstein bull calves. J. Anim. Sci., 79: 2260-2267.

Tozer, P.R. and A.J. Heinrichs, 2001. What affects the costs of raising replacement dairy heifers: A multiples-componet analysis. J. Dairy Sci., 84: 1836-1844.

Velayudhan, B.T., K.M. Daniels, D.P. Horrell, S.R. Hill and M.L. McGilliard *et al*., 2008. Developmental histology, segmental expression and nutritional regulation of somatotropic axis genes in small intestine of preweaned dairy heifers. J. Dairy Sci., 91: 3343-3352.

Weintraub, H., 1993. The MyoD family and myogenesis: Redundancy, networks and thresholds. Cell, 75: 1241-1244.

Wright, W.E., D.A. Sassoon and V.K. Lin, 1989. Myogenin, a factor regulating myogenesis, has a domain homologous to MyoD. Cell, 56: 607-617.