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Changes in the Levels of Insulin-like Growth Factors (IGF-I and IGF-II) in Bovine Milk According to the Lactation Period and Parity

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ABSTRACT : The objectives of this study were to characterize the changes occurring in the levels of insulin-like growth factors (IGF-I and IGF-II) in bovine milk during a one-year lactation period, and to determine the parameters affecting IGF content in bovine milk. Milk was collected individually from lactating Holstein cows (n = 70), and IGF-I and -II levels were determined via radioimmunoassay, using ^{125}I after acid-ethanol treatment. The proximate compositions of the milk samples were determined using a near-infrared milk analyzer. The data were analyzed by the GLM and CORR procedures using SAS software to determine significant differences ($p<0.05$) occurring within groups (dairy farms, lactation periods, season, and parity). We noted an approximately six-fold reduction in the IGF-I concentration (from 2,462.7 to 353.0 ng/ml) and a three-fold drop in the IGF-II concentration (from 929.1 to 365.7 ng/ml) in the bovine colostrum, between 6 h after parturition and 18 h after parturition. IGF-I and -II content, measured at the early, middle, and late stages of lactation did not change significantly throughout the entirety of the lactation period. Interestingly, parity did not significantly affect IGF-I content, but did significantly affect IGF-II content between the primiparous and multiparous cows. We also found there were no significant relationships between IGF-I and total protein content or somatic cell counts ($p<0.05$). (**Key Words :** Insulin Growth Factor-I And -II, Radioimmunoassay, Lactation Period, Parity, Somatic Cell)

INTRODUCTION

There have been several studies concerning Insulin-like growth factors (IGF-I and IGF-II) in bovine milk during the lactation period (Campbell et al., 1989; Collier et al., 1991). Francis et al. (1988) reported that insulin-like growth factors were present in bovine prepartum mammary secretions, and both colostrum and milk. IGF-I and -II have also been isolated in human milks (Baxer et al., 1984), cows (Campbell et al., 1989; Collier et al., 1991), rats (Donovan et al., 1991), pigs (Donovan et al., 1994), and goats (Faulkner, 1999), and chickens (Ko et al., 2005). In bovine colostrums, concentrations of growth factors, including IGF-I, IGF-II, and insulin, decrease substantially with reductions in the levels of milk proteins as lactation proceeds in the early stage (Lee et al., 1995; Vega et al., 1991). This challengers reported that IGF-I levels tended to be highest on 2 wks prepartum (2.949±1.158 ng/ml), and were at their lowest levels in bovine milk at 49 days postpartum (5.0±2.0 ng/ml).

Parity had a slight, but discernable, effect on IGF-I levels in the milk of multiparous animals, at an average concentration of 3 ng/ml, compared with the 2.3 ng/ml detected in primiparous animals (Collier et al., 1991). Campbell and Baumrucker (1989) found that the colostrum of multiparous cows had higher IGF-I concentrations compared with colostrum obtained from first-lactation heifers. Daxenberger et al. (1998) reported that different IGF-I concentrations could be observed in milk samples obtained from cows at their first, second, or third to sixth lactations.

Nutrition is known to be a primary regulator of the

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somatotropin-IGF-I axis, and has also been determined to influence the prepubertal development of the mammary gland. Also, these researchers proposed that these increases in the levels of IGF-I may be attributed to the abrupt termination of milking, and also may be caused by either improved nutritional status or the cessation of IGF removal from circulation by the mammary gland. Growth factors are also believed to play a role in the course of inflammatory processes, including mastitis. (Liebe and Schams, 1998)

As milk samples are easily available in food quality control systems, the objective of this study was to determine the naturally existing IGF-I and -II concentrations in bovine whey, throughout the lactation period. The secondary objective of this study was to identify and quantify the factors that affect the IGF content of bovine milk. These factors included: season, lactation stage, parity, feeding conditions, and the correlation between milk protein and somatic cell counts (SCC), as determined by radioimmunoassay (RIA) for the detection of IGF in milk.

MATERIALS AND METHODS

Animals

Individual milk samples from 70 Holstein dairy cows, which had been managed in two dairy herds in northern Kyoung-ki Province, were assayed for naturally occurring IGF-I contents for one year, between September 2000 and August 2001. Milk yields for one consecutive p.m. and a.m. milking were obtained from a set of milk recordings, which provided information regarding parity and calving dates. Milk samples from p.m. milking were collected in plastic screw-bottles (≥200 ml). The milk constituents (total proteins and somatic cell counts) were determined using a Near-Infrared (NIR) analyzer (Foss, Denmark) in the laboratory in which the milk recording had been conducted. Bovine colostrum samples were collected at three consecutive morning and afternoon milkings for the first two days after calving, at a dairy farm in northern Kyoung-ki Province, Korea. The samples were stored immediately at -20°C and then thawed at room temperature until the next preparation.

The cows were classified according to parity and lactation periods. At farm A, 4 primiparous and 31 multiparous cows were assessed. At farm B, 8 primiparous and 27 multiparous cows were assessed. Lactation periods were classified as early lactation (less than 90 days of lactation), middle lactation (from 91 to 180 days of lactation), and late lactation (more than 181 days of lactation). For the analyses of the IGF-II content, individual milk samples from 10 Holstein dairy cows were collected according to the same farms and periods described above.

Whey preparations

The collected milk samples were conserved using sodium azide (Sigma Chemical Co. St. Louis, MO, USA), in accordance with the regular procedure of milk recording (8 mg per 40 ml milk), and centrifuged for 20 min at 9,000 rpm to separate the fat from the milk. Acid whey supernatant was obtained by adjusting the pH of milk samples to 4.6 with 2 N HCl and centrifuging samples for 15 min at 3,000 rpm. The individual whey samples were labeled and stored immediately at -70°C until the radioimmunoassay was conducted.

Iodination

One microgram of each recombinant human IGF-I and IGF-Ⅱ (Gropep Pty Ltd., Adelaide, Australia) was iodinated to a specific activity of 300-350 mCi/μg protein, using 1 mCi Na ^{125}I (Amersham, Sweden), via the chloramine T method (Lee and Henricks, 1990). The iodinated IGF- I and -II were then purified on a Sephadex G-50 column (Sigma Chemical Co., USA), and aliquots were stored at -20°C until later use. The specific activity per vial was between 16,000 and 20,000 cpm.

IGF-I and -II radioimmunoassays in the whey

The whey samples were assayed for IGF-I and -II content (Etherton et al., 1987). In order to avoid interference from IGF-binding proteins, the acid/ethanol extraction method was used (Campbell et al., 1989). One hundred microliters of milk were mixed with four hundred microliters of acid ethanol (HCl:ethanol = 12.5%:87.5%). This mixture was incubated for 30 min at room temperature and then neutralized using 0.2 ml of 0.855 M Tris-base. The IGFBPs-free supernatant was mixed with 0.1 ml of RIA buffer (30 mM sodium phosphate, 0.02% protamine sulfate, 10 mM EDTA, 0.05% Tween-20, 0.02% sodium azide; pH 7.5) and then incubated with rabbit anti-human IGF-I (or IGF-II) polyclonal antiserum (GroPep Pty., Ltd. Australia; at a final dilution of 1:10,000) and [^{125}I] IGF-I (or IGF-II), and incubated for 16 h at 4°C. After the incubation, 0.1 ml of goat anti-rabbit IgG antibody (Gropep Pty Ltd., Australia) was added, and the mixture was incubated for 1 h, followed by an additional 1 h of incubation with 0.1 ml of normal rabbit serum (Sigma Chemical Co., USA) at 4°C. After the addition of 1 ml of RIA buffer, the tubes were centrifuged for 10 min at 3,000 rpm, at 4°C. The supernatant was aspirated, and the pellets were counted with a gamma-counter (COBRA, Packard Instrument Co., Meriden, CT) for 1 min. All determinations were performed in duplicate.

Statistical analyses

All data were analyzed using the GLM procedure of

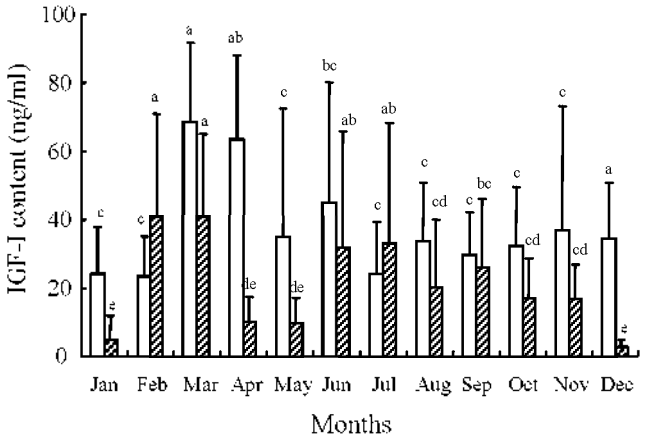

Figure 1. IGF-I concentration throughout an year period in whey at two farms. Open bars located in each month represent content of milk IGF-I in farm A (n = 320), and hatched bars represent in Farm B (n = 325). Different letter superscripts indicate differences at $p<0.05$.

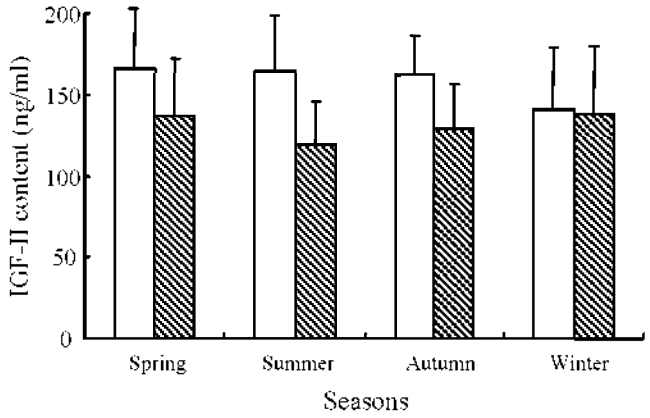

Figure 2. IGF-II concentration throughout seasons in whey at two farms. Open bars represent content of milk IGF-II in Farm A (n = 47), and hatched bars represent in Farm B (n = 57). Different letter superscripts indicate differences at $p<0.05$.

SAS (SAS Institute. 1985). Significant differences ($p<0.05$) between treatment means were assessed using the LSD (least significant difference) method.

Correlation with milk components

The normalized data used in this study was generated using the IGF-I contents in milk (in ng/ml), via the linear regression formula model (Daxenberger et al., 1998). The factors ultimately determined to influence the IGF-I content were somatic cell count (SCC, as thousands per ml) and protein content (%).

RESULTS AND DISCUSSION

Milk IGF-I and IGF-II content over one year of lactation

The whey IGF-I concentrations in the 645 recorded samples over a one-year period at the two farms are shown in Figure 1. At farm A, the IGF-I contents are shown over a wide range, from a mean of 23.7-68.6 ng/ml, with the highest contents observed in March and the lowest contents seen in February. At farm B, however, the mean IGF-I contents were in a range between 2.8-40.7 ng/ml, with the highest levels in March and the lowest levels observed in December. We noted significant differences ($p<0.05$) in monthly variation, but there were no specific seasonal effects on the IGF-I content. Daxenberger et al. (1998) reported that the concentrations of IGF-I in the 5,777 recorded samples were detected over a wide range from 1 to over 83 ng/ml, but found no regional and seasonal effects on IGF-I concentration.

Table 1. IGF-I content in bovine colostrum whey treated with acid ethanol

Individual	Parity	IGF-I in colostral whey after postpartum (ng/ml)		
		6 h	12 h	18 h
A	P	1,548.9	1,112.8	341.8
B	P	1,312.0	319.2	374.5
Means		1,430.5[b]	716.0[b]	358.2[a]
C	M	4,615.4	2,288.7	513.9
D	M	1,300.9	1,192.4	433.2
E	M	2,967.0	319.2	111.2
Means		2,961.1[a]	1,266.8[a]	352.8[a]

[a, b] The values followed by the same letters within a column are not significantly different ($p<0.05$). M and P indicate multiparous and primiparous.

The whey IGF-II concentrations in the 104 samples recorded throughout the season at the two farms are presented in Figure 2. At farm A, the levels of IGF-II were detected over a wide range, from a mean of 140.7-165.6 ng/ml, and at farm B, the mean IGF-II contents occurred in a range between 118.5-137.7 ng/ml. There were no significant seasonal effects on IGF-II concentrations ($p<0.05$). This result was consistent with a previous report, demonstrating that IGF-II levels in serum were remarkably stable (Holly, 1998).

Lactation periods and parities

Table 1 shows the IGF-I contents in individual fractions of bovine colostrum whey. IGF-I content was highest at 6 h after parturition and significantly decreased as the time after parturition increases. The levels of IGF-I in bovine colostrum were reported over a wide range, of 200 ng/ml, 100-450 ng/ml, 2,000 ng/ml, 50-150 ng/ml, and 450-500 ng/ml (Donovan et al., 1991; Donovan et al., 1994). Especially, Campbell and Baumrucker (1989) reported that the colostrum had 10 times higher IGF-I level compared with IGF-I level in milk samples at day 6 postpartum. They also showed that, at parturition, colostrum from multiparous cows exhibited higher IGF-I concentrations than did colostrum from first-lactation heifers. As expected, our

Table 2. IGF-Ⅱ content in bovine Colostrums whey treated with acid ethanol

Individual	Parity	IGF-II in colostral whey after postpartum (ng/ml)		
		6 h	12 h	18 h
A	P	790.4	371.9	291.8
B	P	956.6	652.9	431.7
Means		873.5[b]	512.4[b]	361.7[a]
C	M	945.9	596.7	364.2
D	M	994.5	623.4	370.1
E	M	958.3	543.7	370.8
Means		966.2[a]	587.9[a]	368.4[a]

[a, b] The values followed by the same letters within a column are not significantly different ($p<0.05$). M and P indicate multiparous and primiparous.

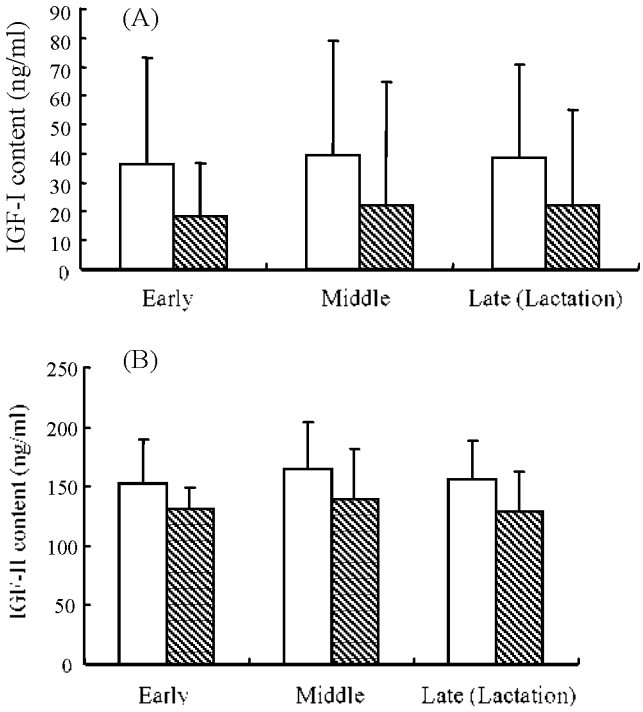

Figure 3. Mean IGF-I (A) and IGF-II (B) concentrations in whey during the entire lactation. Open bars represent content in Farm A, and hatched bars represent Farm B, shown as the mean±SEM.

experiment yielded results similar to these previous studies. These higher IGF-I concentrations in the milk of multiparous cow might be associated with several environmental factors, including the number of receptors per cell, the rate of uptake or metabolism of IGF-I within the mammary epithelial cells. Table 2 shows the IGF-II contents in the individual fractions of bovine colostrum whey. IGF-II levels exhibited a pattern similar to that seen with IGF-I, with the highest levels occurring immediately after parturition.

Relationships between IGF-I or -II content and lactation period were presented in Figure 3. As is shown in these figures, IGF-I and -II content did significantly not change

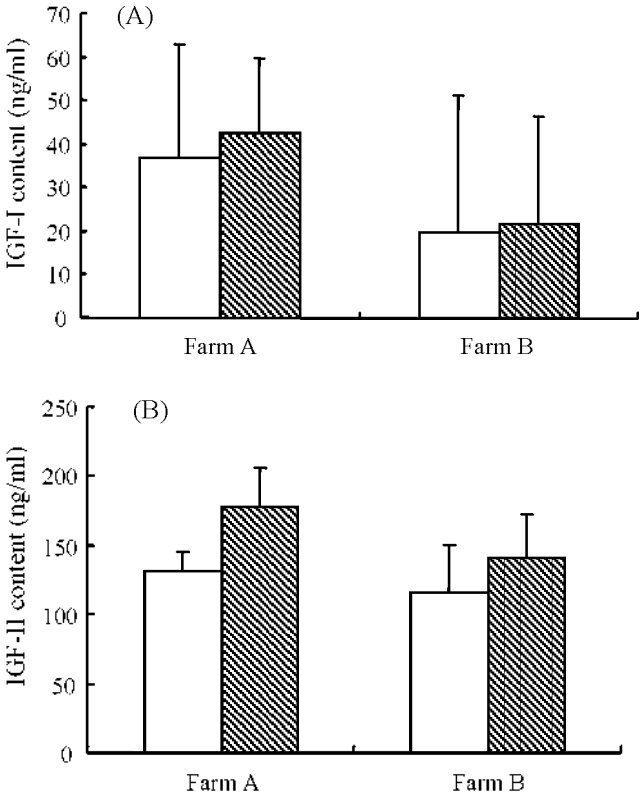

Figure 4. Mean IGF-I (A) and IGF-II (B) concentrations in primiparous and multiparous whey. Open bars represent primiparous IGF-I content, and hatched bars represent multiparous, shown as the mean±SEM.

throughout lactation.

Parity also did not significantly affect the IGF-I concentrations in the milk samples (Figure 4A). On the other hand, the IGF-II contents in the milk of the multiparous animals were significantly higher ($p<0.05$) than those in the milk of primiparous animals at the two farms (Figure 4B).

Relationships between IGF-I levels and milk proteins or somatic cell count

There were no significant relationships between total protein and IGF-I content ($p<0.05$). Mackle et al. (2000) reported that intramammary infusions of both insulin and IGF-I exerted no effect on milk protein yields in dairy cows.

The linear regressions of all 645 milk samples, which illustrate the correlation between the natural values of somatic cell counts (SCC) and IGF-I. The extent of the SCC influence appears similar to that of proteins (R^2 = 0.0086 and 0.0034) but, there were not significant differences between SCC and IGF-I. Liebe and Schams (1998) reported that the significant positive correlation between SCC and IGF-I concentration and suggested that growth factors might play a role in the course of inflammatory processes in mastitic milk. However, such relationship has not been

found in the first grade milk.

In the future, more detailed and validated studies regarding the different reactions of primiparous and multiparous animals should be conducted, and other factors should be assessed with regard to their possible effects on the naturally existing levels of IGF-I.

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