

EFFECT OF NUTRITIONAL STATUS ON CONCENTRATIONS OF INSULIN-LIKE GROWTH FACTOR-I IN MILK FROM HOLSTEIN COWS

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Abstract

The effect of diet on concentrations of milk insulin-like growth factor-I (IGF-I), and their relationships with plasma IGF-I, and with somatic cell count (SCC) were evaluated in two trials. In Trial 1, 32 multi-parous Holstein cows at 4 to 5 wk of lactation received 4 different diets formulated to provide high (H) or low (L) dry matter intake (DMI) with H or L metabolisable energy (ME) density for 5 wk. Dietary treatment did not affect milk IGF-I concentrations, but concentrations were repeatable in individual cows. The intraclass correlation coefficients were 0.78 ± 0.05 and 0.73 ± 0.06 in a.m. and p.m. samples, respectively, when concentrations were measured for 7 consecutive days. The association between milk IGF-I and plasma IGF-I concentration was weaker ($R^2 = 0.140$; $P = 0.035$) than the association between milk IGF-I concentrations and the natural logarithmic value (Ln) of SCC ($R^2 = 0.249$; $P = 0.004$). The effects of body condition score (BCS) at calving and level of grain supplementation in early lactation on IGF-I concentrations in milk were evaluated in Trial 2 with 21 Holstein cows that grazed pasture. The BCS at calving did not affect milk IGF-I concentrations; neither did supplementation with 1 or 6 kg of grain. Milk and plasma concentrations of IGF-I were not associated ($R^2 = 0.002$; $P = 0.830$), whereas the association between milk concentrations of IGF-I and Ln SCC was significant ($R^2 = 0.342$; $P = 0.005$). Concentrations of IGF-I in milk were not a sensitive measure of dietary changes in lactating pasture-fed Holstein cows.

Keywords: Insulin-like growth factor-I, milk, dietary factors, somatic cell count

EFFET DU STATUT NUTRITIONNEL SUR LES CONCENTRATIONS D'INSULINE DE CROISSANCE DANS LE LAIT DE VACHES HOLSTEIN

Résumé

L'effet du régime sur les concentrations d'insuline de croissance dans le lait et leurs relations avec le nombre des cellules somatiques (CCS) ont été évalués dans deux essais. Dans l'essai 1, 32 vaches Holstein multipares de 4 à 5 semaines de lactation ont reçu 4 régimes différents formulés pour fournir de haute (H) ou faible (L) ingestion de matière sèche (IMS) avec une densité d'énergie métabolisable H ou L pendant 5 semaines. Le traitement alimentaire n'a pas affecté les concentrations d'insuline de croissance dans le lait, mais les concentrations étaient les mêmes chez les vaches individuelles. Les coefficients de corrélation intra classe de 0.78 ± 0.05 et 0.73 ± 0.06 respectivement le matin et le soir ont été calculés quand les concentrations ont été mesurées pendant 7 jours consécutifs. La relation entre la concentration d'insuline et le nombre de cellule somatique était plus faible ($R^2 = 0,140$, $P = 0,035$) que celle sa valeur logarithmique naturelle ($R^2 = 0,249$, $P = 0,004$). Les effets de la note d'état corporel au vêlage et le niveau de suppléments des céréales en début de lactation sur les concentrations d'insuline dans le lait ont été évalués lors de l'essai 2 avec 21 vaches Holstein. Le la note d'état corporelle au vêlage n'a pas affecté la concentration d'insuline pas plus que la suppléments

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avec 1 ou 6 kg de céréales. Les concentrations d'insuline de croissance dans le lait ne sont pas une mesure sensible de changements alimentaires en lactation des vaches Holstein nourris au pâturage.

Mots-clés: Concentration de l'insuline de croissance le lait, le régime alimentaire des facteurs, le nombre de cellules somatiques

Introduction

Insulin-like growth factor-I (IGF-I) is a small peptide hormone of approximately 7kDa molecular mass (Hwa *et al.*, 1999) that plays important roles in mammary gland development, differentiation and milk production in ruminants (Akers *et al.*, 2000). Its primary source is the liver (Rosen and Pollak, 1999) and it may exert an endocrine effect on the mammary gland. There is also evidence for local synthesis and secretion of IGF-I and IGF binding proteins (IGFBPs) by the mammary gland of lactating cows (Sharma *et al.*, 1994). The relative contributions of systemic as compared to locally synthesized IGF-I to bovine mammary differentiation, growth and lactogenesis have not been resolved. Similarly, the contribution of each of these two sources to IGF-I concentrations in milk is unclear (Baumrucker and Erond, 2000).

The concentrations of IGF-I in milk vary with stage of lactation in dairy cows. They decline at the approach of parturition, remain low in early lactation and then increase gradually through lactation (Sejrsen *et al.*, 2001). There are some reports on the pattern of changes in milk IGF-I concentrations in dairy cows during early lactation (Skaar *et al.*, 1991; Vega *et al.*, 1991; Sejrsen *et al.*, 2001). These studies have been conducted using dairy cows fed completely balanced, total mixed rations (TMR). Limited information exists on the relationship between plasma and milk concentrations of IGF-I.

Plasma concentrations of IGF-I are affected by nutritional status (Thissen *et al.*, 1994). These changes can be used as a sensitive monitor of energy balance in dairy cows fed TMR during the postpartum period (Beam and Butler 1999). They are also a sensitive measure of dietary changes in cows in pasture-based systems during early lactation (Obese *et al.*, 2008b). There

is a lack of information on nutritional effects on milk concentrations of IGF-I, as well as, the associations between milk and plasma concentrations of IGF-I among cows in pasture-fed dairy herds.

The objectives of the present study were to evaluate: (i) the effects of varying either level of DMI or ME density during early lactation on milk concentrations of IGF-I; (ii) the effects of BCS at calving and different levels of grain feeding in early lactation on milk concentrations of IGF-I; (iii) the day- to-day variation in IGF-I concentrations in milk of individual cows; and (iv) the relationships between milk concentrations of IGF-I with plasma concentrations, and milk SCC.

Materials and Methods

Animals and Experimental Procedures

Two studies were conducted in Victoria, Australia in 2000 and 2001. Trial I was conducted at the Ellinbank Dairy Research Centre, Victorian Department of Primary Industries during August and September, 2000 (Obese *et al.*, 2008b). Thirty-two multiparous Holstein cows 4 to 5 wk postpartum were randomly assigned to one of four treatments receiving different diets. Briefly, these diets were formulated to provide high (H) or low (L) DMI with H or L ME density for 5 wk (comprising a 3- wk adaptation period followed by 2 wk of intensive sampling). The treatments were LL: 16.6 kg of DMI and 174 MJ of ME; HL: 17.3 kg of DMI and 181.1 MJ of ME; LH: 15.4 kg of DMI and 183.1 MJ of ME; HH: 17.9 kg of DMI and 213.3 MJ of ME. The diets comprised freshly cut ryegrass-clover pasture, meadow hay and pelleted barley grain to achieve the two different levels of DMI and ME. The cows were milked at 06:00 and 15:00 h daily before being individually offered their allocated diet at 09:00 h and 16:00 h for 5 h. Water was available between the feeding and milking times.

Trial 2 was conducted between June and November 2001, at the Kyabram Dairy Centre, Victorian Department of Primary Industries. Twenty-one Holstein cows were managed to calve in three BCS groups of 4 (3.5 to 4.5), 5 (4.6 to 5.5) or 6 (5.6 to 6.5) on an 8-point score (Earle, 1976). There were 9, 4 and 8 cows in BCS groups 4, 5 and 6 respectively. The cows grazed pasture after calving at a pasture allowance of 35 to 40 kg DM/cow/day and were provided with either 1 or 6 kg of wheat grain energy concentrate daily for 10 wk (Stockdale, 2004). The concentrates were individually fed immediately after each milking before the cows returned to pasture to graze. They were milked at 06:00 and 15:00 h daily, and water was available only at milking times.

Milk and Blood Sampling and Assays

Milk samples were hand drawn (fore strippings) weekly from each cow during the 5-wk study in Trial 1 into 10 mL plastic vial tubes at 06:00 h. Samples were also collected daily for 7 consecutive days at 06:00 h and 16:00 h in Wk 4 to estimate within day variation in milk concentrations of IGF-I. Each sample was kept on ice and centrifuged shortly after collection at 1500 g for 15 min at 4°C. The fat was removed after centrifugation and the “fat-free” samples stored at -20°C until assayed for IGF-I concentration.

Milk samples were collected from the 21 cows in Trial 2 at 5 and 10 wk of lactation. The samples were processed as for Trial 1. Blood samples were also taken weekly from the coccygeal vessels of cows into 10 mL heparinized vacutainer tubes in both trials 1 and 2. The samples were centrifuged shortly after collection at 1500 x g for 15 min at 4°C. Plasma was aspirated and stored at -20°C for subsequent analysis for IGF-I concentration.

The IGF-I concentrations in milk and plasma were measured with the DSL-10-2800 ACTIVE TM Enzyme-linked Immunosorbent assay (ELISA) commercial kit (Diagnostic Systems Laboratories Inc, Webster, TX, USA) which had been validated against an IGF-I radioimmunoassay (RIA) (Obese et al., 2008a). Validation results showed low coefficient of

variation (CV) values (intra-assay and inter-assay CVs for the milk IGF-I assay were 2.0 % and 3.5 %, and for the plasma IGF-I assay were 2.3 % and 4.1 %). Minimum detection limit was 10 ng/ mL for the milk and plasma assays.

The SCC was determined in weekly milk samples in both trials using a Milkoscan (Foss Electric, Denmark).

Statistical Analyses

The IGF-I concentrations in milk and plasma, and SCC of individual cows in each week were averaged over the 5-wk period of the study in Trial 1 and values used in the evaluation of diet on milk IGF-I, plasma IGF-I and SCC. The above data were analyzed by the Kruskal-Wallis One Way analysis of variance on ranks in SPSS version 11.5 (SPSS Inc., 2002). The Kruskal-Wallis one-way analysis of variance is a non-parametric method for testing equality of population medians among groups. It is identical to a one-way analysis of variance with the data replaced by their ranks (measurement observations are converted to their ranks in the overall data set). It is an extension of the Mann-Whitney U test (McDonald, 2009). The association between IGF-I concentrations in a.m. and p.m. milk samples from the 7-day daily sampling period during Wk 4 was estimated using linear regression analysis. The day-to-day variation in IGF-I concentrations in a.m. or p.m. samples over the same 7-day measurement period was evaluated using intraclass correlations (ICC; Snedecor and Cochran, 1980) and Kendall's coefficient of concordance (SPSS Inc., 2002). Values for milk and plasma concentrations of IGF-I and SCC for the individual cows (n = 21) at 5 and 10 wk of lactation in Trial 2 were averaged and the data used in assessing the effect of BCS at calving (4, 5 and 6) on milk and plasma concentrations of IGF-I, and SCC. Analyses were also by the Kruskal-Wallis One-way analysis of variance on ranks. The effect of level of grain feeding on these three variables was by Mann-Whitney One-way analysis of variance on ranks in SPSS v 11.5. Data on SCC were transformed to natural logarithmic values (Ln) and linear regressions

were used to assess the relationships between milk and plasma concentrations of IGF-I and Ln SCC.

Results

Milk Concentrations of IGF-I in Trial 1

The milk IGF-I concentrations were low compared to plasma IGF-I concentrations. The IGF-I concentrations in weekly milk samples in the 4 dietary groups generally peaked at d14, followed by declines of varying magnitude (Figure 1). The average concentrations of milk IGF-I for the 32 cows in the 4 treatment groups were only 7.5 ± 1.8 and 5.9 ± 1.7 ng/mL at d 0 (the beginning) and d35 (the end) respectively, compared to corresponding values in plasma of 58.1 ± 4.6 and 60.4 ± 4.3 ng/mL. Dietary treatment did not affect milk concentrations of IGF-I or SCC when ranked in cows, while the plasma concentrations of IGF-I were significantly affected by diet (Table 1). Cows in the HH-dietary group had the highest mean ranking, while those in the LL-dietary group had the lowest.

Concentrations of IGF-I in a.m. and p.m. milk samples in Trial 1

The intraclass correlation (used as a measure of the proportion of the total variance that was due to between cow variance) was high and significant for a.m. and p.m. samples, as well as, the average for a.m. and p.m. samples (0.73 to 0.78; Table 2). The Kendall's coefficient of concordance calculated as a measure of agreement among rankings of the cows over each of the 7 d of milk IGF-I were also high (a.m. = 0.76; $P < 0.001$; and p.m. = 0.71; $P < 0.001$). There was a strong association (Table 3) between IGF-I concentrations in a.m. and p.m. samples on each of the 7 d as well as the overall mean for each animal over the 7-d period. High milk IGF-I concentrations in some a.m. and p.m. samples were associated with high SCC ($> 100,000$) in individual cows.

Milk and Plasma Concentrations of IGF-I, and SCC in Trial 2

Body condition scores at calving were not associated with differences in the concentrations of milk and plasma IGF-I, or with SCC when cows were ranked. Level of grain feeding did not influence IGF-I concentration in milk (Table 4). By contrast, cows supplemented with 6 kg of grain per day, had higher mean ranking for plasma IGF-I than those on the 1 kg grain supplement (Table 4). Level of grain feeding did not affect the mean rankings for SCC.

Relationships among concentrations of IGF-I in milk and plasma, and SCC

The relationships between milk and plasma concentrations of IGF-I were weaker than those between milk IGF-I and Ln SCC in both trials (Table 5).

Discussion

Neither the IGF-I concentrations in milk nor SCC were influenced by dietary treatments used in Trials 1 and 2 in contrast to plasma concentrations of IGF-I. Concentrations of IGF-I in milk samples were not affected by BCS at calving (Trial 2). Information on the nutritional effect on milk IGF-I concentrations in the postpartum period is limited, but concentrations of IGF-I in mammary gland extracts were not influenced by level of feeding in prepubertal Holstein heifers (Weber *et al.*, 2000).

The mean concentrations of IGF-I in milk for the dietary treatments in the present study were within the range of concentrations previously reported in milk from Holstein cows in other studies (Daxenberger *et al.*, 1998; Taylor *et al.*, 2004).

Although milk IGF-I concentrations differed among cows, the concentrations were repeatable for individual cows. This consistency contributed to the high ICC of 0.78 for IGF-I in a.m. and 0.73 for p.m. milk samples, as well as the high Kendall's coefficients of concordance of 0.76 for IGF-I in a.m. and 0.71 in p.m. samples. The high association with respect to milk IGF-I concentrations in a.m. and p.m. milk samples on each day of the 7-day sampling may partly

Table 1: Effect of diet on ranked milk and plasma IGF-I concentrations and somatic cell count in weekly samples in Trial I (mean \pm se)

Group	Na	Milk IGF-I		Plasma IGF-I		SCC ^b	
		Concentrations (ng/mL)	Mean Rank	Concentrations (ng/mL)	Mean Rank	Concentrations (ng/mL)	Mean Rank
LL ^c	8	6.4 \pm 2.1	14.69	44.7 \pm 7.5	10.88	87.98 \pm 51.28	16.75
HL ^d	8	9.3 \pm 4.9	14.63	51.7 \pm 5.9	13.50	240.71 \pm 191.1	20.25
LH ^e	8	9.2 \pm 3.9	16.69	64.2 \pm 10.3	17.13	101.23 \pm 59.82	15.88
HH ^f	8	11.0 \pm 3.3	20.00	74.6 \pm 7.9	24.50	49.27 \pm 23.05	13.13
P ^g			0.629		0.023		0.502

^anumber of animals in each treatment group; ^bsomatic cell count; ^chigh DMI and high ME; ^dhigh DMI and low ME; ^elow DMI and high; ^flow DMI and low ME; ^gProbability value

Table 2: Intraclass correlations at exact 95% confidence intervals for milk IGF-I concentrations in a.m. and p.m. milk samples during a 7-day consecutive sampling period in Trial I

Time	Intraclass correlation	P-value	R ²	Exact 95% confidence intervals
a.m	0.78 \pm 0.05	<0.001	0.809	0.68 to 0.87
p.m	0.73 \pm 0.06	<0.001	0.764	0.62 to 0.83
Average. a.m. and p.m. For individual cows	0.77 \pm 0.05	<0.001	0.796	0.67 to 0.86

Table 3: Association between a.m. and p.m. milk IGF-I concentrations in milk samples for 7 consecutive days in Trial I (mean \pm se)

Day	Milk IGF-I (ng/ mL)		Intercept \pm se	Slope \pm se	R ²
	a.m.	p.m.			
1	11.7 \pm 2.0	11.1 \pm 2.1	0.548 (\pm 0.587)	1.003 (\pm 0.037)	0.961***
2	7.9 \pm 2.0	6.6 \pm 2.0	1.433 (\pm 0.529)	0.979 (\pm 0.041)	0.951***
3	4.9 \pm 1.6	4.0 \pm 1.6	0.343 (\pm 0.550)	1.130 (\pm 0.065)	0.911***
4	7.3 \pm 2.1	6.6 \pm 2.0	0.560 (\pm 0.580)	1.013 (\pm 0.044)	0.946***
5	7.7 \pm 2.0	6.5 \pm 2.0	1.703(\pm 0.722)	0.923 (\pm 0.055)	0.904***
6	7.4 \pm 2.3	6.2 \pm 2.0	0.506 (\pm 0.463)	1.108 (\pm 0.036)	0.970***
7	9.1 \pm 2.2	8.2 \pm 2.4	1.629 (\pm 0.698)	0.905 (\pm 0.045)	0.931***
Overall	8.00 \pm 1.9	7.0 \pm 1.8	0.630 (\pm 0.314)	1.045 (\pm 0.026)	0.982***

*** P <0.001

reflect the effect of IGF-BPs prolonging the half-life of IGF-I in milk (Hadsell *et al.*, 1993). This suggests that IGF-I concentrations could be measured in milk samples recovered at any time of the day on a weekly basis. A similar suggestion was made for taking samples to measure plasma concentrations of IGF-I, as the overall intraclass correlation for plasma samples obtained from the cows enrolled in Trial I was 0.77 and the Kendall's coefficient of concordance was 0.84 (Obese *et al.*, 2008b).

The observed concentrations of IGF-I in milk were low compared with those in plasma samples from the same cows in both trials. Low milk IGF-I concentrations compared to plasma levels in the postpartum period has been reported for dairy cows (Skaar *et al.*, 1991; Vega *et al.*, 1991; Taylor *et al.*, 2004). The different patterns of milk and plasma concentrations of IGF-I as seen in this study suggest that, the regulation of circulating IGF-I differs to that in the mammary gland. Milk IGF-I levels may not simply reflect changes in

Table 4: Effect of diet on ranked milk and plasma IGF-I concentrations, and SCC in weekly samples in Trial 2 (mean \pm se)

Group BCS or Supp. level	N ^a	Milk IGF-I		Plasma IGF-I		SCC ^b	
		Concentration	Mean Rank (ng/ mL)	Concentration	Mean Rank (ng/ mL)	1000/ ml	Mean Rank
BCS effect							
4	9	8.7 \pm 4.2	7.78	66.1 \pm 7.6	13.33	31.61 \pm 11.26	8.89
5	4	13.5 \pm 3.5	12.50	51.5 \pm 8.0	9.25	129.50 \pm 58.02	16.25
6	8	26.0 \pm 8.2	13.88	53.7 \pm 8.2	9.25	134.56 \pm 90.89	10.75
P-value			0.112		0.328		0.141
Supplement effect							
1	13	15.7 \pm 4.7	11.62	47.2 \pm 4.2	7.69	107.35 \pm 57.81	11.73
6	8	16.9 \pm 8.0	10.00	77.1 \pm 6.4	16.38	60.37 \pm 30.36	9.81
P-value			0.562		0.002		0.491

^anumber of animals in each treatment group.

^bsomatic cell count.

Table 5: Relationship of milk IGF-I concentration with plasma IGF-I concentration, and Ln SCC in weekly samples in Trials 1 and 2

Trial	Plasma IGF-I				Ln SCC ^a			
	intercept (\pm se)	slope (\pm se)	R2	P-value	intercept (\pm se)	slope (\pm se)	R2	P-value
1	-0.491(\pm 4.559)	0.159(\pm 0.072)	0.140	0.035	-5.340(\pm 4.767)	8.650(\pm 2.743)	0.249	0.004
2	18.505(\pm 11.403)	-0.040(\pm 0.183)	0.002	0.830	-9.502(\pm 8.727)	16.716(\pm 5.316)	0.342	0.005

^anatural logarithmic value of somatic cell count.

plasma IGF-I concentrations in the pasture-based management system used in Trials 1 and 2. This result is supported by other studies using more intensive management of dairy herds where increases in plasma concentrations of IGF-I occurred with concurrent decreases in milk IGF-I during early lactation in Holstein cows (Skaar *et al.*, 1991; Vega *et al.*, 1991; Taylor *et al.*, 2004). Although reasons for the weak association between plasma and milk IGF-I concentrations in this study were not apparent, they could be due to the influence of other factors in the local environment of the mammary gland including high SCC. It is remarkable that an individual cow may have consistent but contrastingly different concentrations of IGF-I in samples of milk and plasma obtained at consecutive milkings or on consecutive days.

Daxenberger *et al.*, (1998) found that SCC concentrations can influence milk concentrations of IGF-I in dairy cows. In the present study, high SCC were associated with

high milk IGF-I concentrations in some cows. For example, a cow with subclinical mastitis in Trial 1 had a high mean SCC (489,000/ mL) and a concurrently high mean milk IGF-I concentration of 40.7 ng/mL. Similarly, a cow in Trial 2 had a high mean SCC (274,000/ mL) and a high mean IGF-I concentration of 56.5 ng/mL in its milk. The release of Somatotropin during experimentally induced *E. coli* mastitis in periparturient cows has been reported (Shuster *et al.*, 1995; Burvenich *et al.*, 1999). The concentration of IGF-I in plasma did not change, whereas milk IGF-I increased significantly in milk from infected glands (Shuster and Kehrl, 1995). This increase was attributed partly to leakage of plasma IGF-I into the mammary gland and to de novo synthesis in mammary epithelial cells (Shuster *et al.*, 1995, Burvenich *et al.*, 2007). High IGF-I concentrations in milk may be associated with tissue repair or the healing process, but are equally likely to be due to leakage from blood as seen with associated

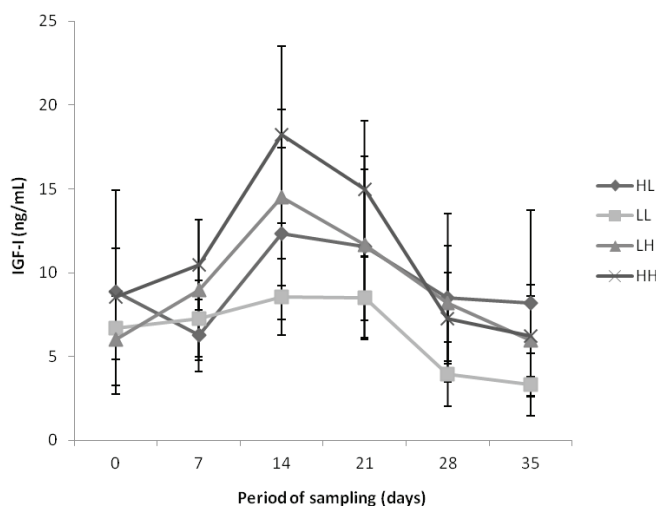


Figure 1: IGF-I concentrations (mean \pm se) in milk samples from 32 cows fed 4 diets (n=8 cows per dietary treatment; HH=High DMI and high ME; HL= high DMI and low ME; LH= low DMI and high ME ; LL= low DMI and low ME).

changes in blood constituents like sodium and albumin that are associated with clinical mastitis or sub-clinical mastitis (Burvenich, 1983). Based on the results from the two trials in the current study, the concentration of IGF-I in a composite milk sample from a herd is likely to be affected by the prevalence of high SCC associated with udder infections. This should be taken into account in studies in humans to measure associations between dietary and serum concentrations of IGF-I (Crowe *et al.*, 2009).

Conclusion

Concentrations of IGF-I in milk were generally low and variable between individual cows and were not affected by diet or BCS at calving. They were consistent for individual cows for milk samples obtained at consecutive milkings and on consecutive days. Associations between plasma and milk IGF-I concentrations were less than the associations between SCC and milk IGF-I concentrations. Milk IGF-I concentrations are not a sensitive measure for monitoring dietary changes and energy balance in pasture-fed Holstein cows. Plasma concentrations of IGF-I provided stronger relationships. The significance of IGF-I as a potential indicator of physiological status may be that IGF-I in milk reflects a

different aspect of partitioning of nutrients to that reflected by IGF-I in plasma. However, milk IGF-I may be a useful monitor of udder health during lactation.

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