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Serum and milk leptin concentrations in Thai native beef cows (*Bos indicus*) affect their offspring's body weight

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Abstract

It has been proposed that maternal serum leptin provides a link between milk leptin and offspring growth and development. Moreover, it may play a role in regulating offspring appetite and food intake in early life. Serum leptin concentration has never been investigated in beef cows, and the link between milk leptin and offspring growth has never been studied. The aims were to investigate whether changes in serum and milk leptin concentrations during postpartum in Thai native beef cows (*Bos indicus*) affect their offspring's body weight and serum leptin concentration. Cow blood and milk samples were collected during 42 days after calving. Calf blood samples were collected, and their body weight was also measured. Serum and milk leptin concentrations were determined using enzyme-linked immunosorbent assay (ELISA). In beef cows, serum and milk leptin concentrations were gradually decreased after postpartum. There was a strong positive correlation between serum and milk leptin (r^2 = 0.73; P<0.01). Both serum and milk leptin concentrations had a strong negative correlation with calf body weight (r^2 = -0.71; P<0.01 and r^2 = -0.76; P<0.01, respectively). Interestingly, there were no correlations noted between calf serum leptin and calf body weight (r^2 = -0.24), between calf serum leptin and cow serum leptin (r^2 = 0.13), and between calf serum leptin and cow milk leptin (r^2 = -0.06). These findings suggest that, in beef cows, maternal serum and milk leptin are related and may be involved in the regulation of their offspring's body weight.

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1. Introduction

Serum leptin is a hormone made by adipose cells that helps to regulate energy balance in mammals. Leptin has also been found in the milk of mammals. The relation between maternal serum leptin and milk leptin as well as the relation between the mother's milk leptin and offspring growth and development have been investigated in humans [1-4], rodents [5], non-ruminants [6-7], and ruminants [8-9]. However, the results are rather contradictory among species.

In humans, maternal serum leptin provides a link between milk leptin and infant growth and development [10] and may also play a role in regulating infant appetite and food intake in early infancy [3]. It has been found that leptin may play a role in the regulation of gastrointestinal functions, as the presence of leptin receptors has been found in gastric and intestinal epithelial cells of both humans and rats [1]. To the best of our knowledge, serum leptin concentration has never been investigated in beef cows, and also the link between milk leptin and offspring growth has never been studied.

Cattle have been domesticated and raised for centuries to provide milk, meat, hides, and to work as draft animals. There are two major species of beef cattle that are commonly raised for consumption, including *Bos indicus* and *Bos taurus*. Thai native cattle, belonging to *Bos indicus*, are characteristically hardy in the tropical environment, intermediate in size among beef breeds, and heat tolerant. They are usually domesticated for draft purposes, and infrequently slaughtered for their meat. On the other hand, *Bos taurus* has been introduced from European countries. Their productivity is higher when compared to that of *Bos indicus* [11]. In Thailand, crossbreeding between *Bos indicus* and *Bos taurus* is popular for commercial beef production. This can be one of factors that leads Thai native cattle to become endangered.

Although there is a wealth of data on the economic traits, nutrient utilization, growth, health, and environmental adaptation in Thai native cattle, the physiological information on reproduction of Thai native cattle remains scarce. The aims of this study were therefore to study changes in serum and milk leptin in beef cows during lactation and to investigate whether maternal leptin plays a role in regulation of their offspring's body weight. This knowledge will provide a better understanding of how serum and milk leptin in Thai native beef cows affect offspring's body weight, which leads to a better management for improving their productivity in the future.

2. Materials and Experiment

2.1 Animal care

All procedures conducted in this study were approved by the ethics committee (Institutional Animal Care and Use Committee) of Suranaree

University of Technology (SUT). The experiments were performed at SUT Farm, Nakhon Ratchasima, Thailand. Twelve multiparous female Thai native beef cows (*Bos indicus*) used in this study were obtained from the livestock breeding station, Chaiyaphum, Thailand. All the cows were Esan cattle, ranging in age from 5 to 7 years and weighing 250 to 300 kg.

2.2 Experimental design

The cows were housed in a tie stall barn and reared under the same conditions throughout the study. They were fully fed with both roughage and concentration twice daily. The cows were estrous synchronized and inseminated artificially with frozen semen from a Thai native bull (*Bos indicus*). The protocol of artificial insemination (AI) was followed as indicated in the guideline of AI (Department of Livestock Development, Thailand). Pregnancy diagnosis was performed by rectal palpation 60 days after AI using a skilled practitioner licensed by the Department of Livestock Development, Thailand.

Day 0 was defined as the day of calving. Blood and milk samples were collected on days 1, 3, 5, 7, 14, 21, 28, 35, and 42 after calving. For blood sample collection, the samples were centrifuged at 1500 rpm for 20 minutes. Serum was separated and stored at -20°C until quantitative analyses were performed. Colostrum and milk were analyzed for their compositions including fat (%), protein (%), and lactose (%). Skim milk or plasma phase was stored at - 20°C until quantitative analyses were performed.

Blood samples (2 mL) were collected from the calf jugular vein at the same time points and with the same protocol as with their mothers. Calf body weight (kg) was also measured on the same day of blood sampling by using the formula heart girth^2 (inches) x body length (inches) x 0.45/ 300 = animal weight in kilograms.

Since bovine leptin shares approximately 87% homology with human leptin [16], a human leptin enzyme- linked immunosorbent assay (ELISA) kit (RayBiotech, Inc., Norcross, GA, USA) was used to measure serum and milk leptin concentrations in this study. Protocols given by the manufacturers were strictly followed in all analyses. According to the manufacturer's information, the minimum detectable dose of human leptin was determined to be 2 pg/mL. Minimum detectable dose was defined as the analytic concentration resulting in an absorbance that is two standard deviations higher than that of the blank (diluent buffer).

2.3 Statistical analysis

Experiments were performed in triplicate and repeated three times. The results were analyzed using a one-way analysis of variance (ANOVA) followed by the Dunnett post-hoc test. The significance level was set at $P<0.05$. The SPSS, a statistical analysis program, Version 15 (SPSS Inc, USA), was used. Correlations between various parameters were calculated using the Pearson product moment correlation coefficient.

3. Results and Discussion

Table 1 shows the mean concentrations of leptin in beef cow serum, beef cow milk, offspring serum, as well as offspring body weight. Table 2 shows the correlation matrix among cow serum leptin, cow milk leptin, milk components, calf serum leptin, and calf body weight.

Both beef cow serum and milk leptin concentrations were decreased as days of postpartum were increased. Leptin concentrations in serum were significantly higher than those in milk ($P<0.01$). There was a narrow range of leptin concentrations in serum (1.23-1.78 ng/mL) and milk (0.44-1.87 ng/mL) during the 42 days postpartum. The mean value of leptin concentrations in serum and milk was 1.52±0.17 ng/mL and 0.77±0.48 ng/mL, respectively, $n = 12$. Leptin concentration in serum was positively correlated with that in milk ($r^2 = 0.73$; $P<0.01$). A study done in goats reported that leptin concentrations in serum were increased, whereas leptin concentrations in milk were decreased, by day postpartum [10]. In the present study, in beef cattle, both serum and milk leptin concentrations were decreased by day postpartum. Furthermore, there was a relation between leptin in serum and in milk. This may suggest that leptin is transferred from the blood circulation to mother's milk, as has been reported in humans [1].

It is interesting to note that the concentrations of leptin in beef cow milk were decreased, with the greatest milk leptin concentrations occurring in colostral milk samples (days 1-3 postpartum). Leptin concentration was 55% lower in mature milk (day 3) than colostrum (1.87 vs. 0.85 ng/mL; $p < 0.01$). These results are similar to those reported in goats [8], sheep [9], mares [12], pigs [13], humans [14], and bovine [15]. Our findings about changes in milk leptin pattern with the peak in colostrum supported the idea that the peak in milk leptin occurs when neonates are best able to absorb large proteins through the gastrointestinal tract. It is therefore impossible that leptin may play a role in the modulation of neonatal gastrointestinal functions, as also suggested by the studies in other livestock species [6-9], humans [4], and rodents [16]. Further investigation of concentration of leptin localized in gastric or intestinal mucosa in calves would be useful to understand such a mechanism, as has been done in humans [17].

It has been reported that, in rodent species, neonatal leptin surge is supplied by exogenous leptin (e.g., mother's milk) and that a leptin surge in circulation after suckling could be found [1, 5-7]. Unfortunately, no change in leptin concentrations in calves from birth until 42 days after birth was observed in this study. This may due to the collection time of the blood samples is involved.

Calf body weight was significantly increased by day and was negatively correlated with beef cow leptin concentration in both serum ($r^2 = -0.71$; $P<0.01$) and milk ($r^2 = -0.76$; $P<0.01$).

Interestingly, there was no change in calf serum leptin. The mean value of leptin concentration in serum was 1.88±0.08 ng/mL (range: 1.75-1.98 ng/mL; n = 12). There were also no relations noted between calf serum leptin and calf body weight (r^2 = -0.24), between calf serum leptin and cow serum leptin (r^2 = 0.13), and between calf serum leptin and cow milk leptin (r^2 = -0.06). Taken together, this result confirms that serum and milk leptin in Thai native beef cows affects their offspring body weight.

As mentioned, in this study both serum and milk leptin of beef cows were negatively correlated with their offspring's body weight. A negative relationship between maternal milk leptin concentration and neonatal weight gain has also been reported in humans [2-4]. These findings suggest that changes in the leptin content of the mother's milk during lactation might play a role in the regulation of body weight in neonates in early life. However, in does, it was reported that leptin presented in milk and serum of does was weakly correlated with doe body weight and body condition but not with kid body weight [8].

Table 3 shows milk compositions. Milk protein and lactose gradually were decreased by day postpartum. However, after parturition, milk fat gradually was increased and reached peak around day 3 postpartum and gradually decreased by day afterward. As shown in Table 2, it was found that cow serum leptin was positively correlated with milk fat (r^2 = 0.59; $P<0.05$). This supported the findings of Feuermann and colleagues, who reported that leptin affected prolactin action on milk protein and fat synthesis in the bovine mammary glands [18]. In this study, however, cow serum leptin was not correlated with milk lactose and milk protein (r^2 = 0.42 and r^2 = 0.42, respectively). Interestingly, there were positive relations between cow milk leptin and milk protein (r^2 = 0.81; $P<0.01$) and milk lactose (r^2 = 0.81; $P<0.01$). However, there were no relations between cow milk leptin and milk fat (r^2 = 0.32). It should be noted that there were no relations between calf body weight and milk fat, milk protein, and milk lactose (r^2 = -0.55, r^2 = -0.55, and r^2 = -0.52, respectively). This was also the case for calf serum leptin. Thus, there were no relations between calf serum leptin and milk fat, milk protein, and milk lactose (r^2 = 0.12, r^2 = -0.01, and r^2 = 0.01, respectively).

Table 1 Mean concentrations of leptin level and calf body weight. Blood and milk samples were collected on days 1, 3, 5, 7, 14, 21, 28, 35, and 42 after calving, and calf body weights were determined on the same day. [a-f] Mean within a column with different letters are different (P<0.05). Data are expressed as the mean ± SD.

Leptin Levels				
Day (After calving)	**Cow Serum (ng/mL) *n = 12***	**Cow Milk (ng/mL) *n = 12***	**Calf Serum (ng/mL) *n = 12***	**Calf Body Weight (kg) *n = 12***
1	1.70±0.62	1.87±0.23[a]	1.87±0.25	17.50±3.30[ef]
3	1.78±0.65	1.66±0.27[ab]	1.83±0.34	18.44±3.37[ef]
5	1.70±0.60	0.85±0.08[cd]	1.93±0.31	20.10±3.47[def]
7	1.74±0.47	0.82±0.13[cde]	1.86±0.20	23.88±3.44[de]
14	1.54±0.34	0.59±0.14[de]	1.98±0.18	27.25±4.20[cd]
21	1.46±0.43	0.66±0.12[de]	1.98±0.28	32.38±4.14[bc]
28	1.39±0.34	0.54±0.15[de]	1.98±0.26	36.38±5.48[abc]
35	1.33±0.18	0.49±0.17[de]	1.86±0.39	41.25±5.37[ab]
42	1.23±0.13	0.44±0.18[e]	1.75±0.36	45.00±7.33[a]

Table 2 Correlation matrix among cow serum leptin, cow milk leptin, milk components, calf serum leptin, and calf body weight.

	Cow serum leptin	**Cow milk leptin**	**Milk fat**	**Milk protein**	**Milk lactose**	**Calf body weight**	**Calf serum leptin**
Cow serum leptin	1.00						
Cow milk leptin	0.73**	1.00					
Milk fat	0.59*	0.32	1.00				
Milk protein	0.42	0.81**	-0.23	1.00			
Milk lactose	0.42	0.81**	-0.20	.99**	1.00		
Calf body weight	-0.71**	-0.76**	-0.55	-0.55	-0.52	1.00	
Calf serum leptin	0.13	-0.06	0.12	-0.01	0.01	-0.24	1.00

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Table 3 Milk compositions

Day (After calving)	**Milk Fat (%)** ***n = 12***	**Milk Protein (%)** ***n = 12***	**Milk Lactose (%)** ***n = 12***
1	0.56±0.18	6.38±0.19	9.22±0.38
3	7.01±1.05	3.72±0.28	5.32±0.28
5	4.83±0.62	3.43±0.18	4.68±0.30
7	3.62±0.62	3.55±0.05	4.52±0.22
14	4.08±1.88	3.59±0.12	5.23±0.41
21	2.85±1.43	3.46±0.15	4.60±0.36
28	1.83±0.26	3.29±0.17	4.49±0.22
35	1.76±0.24	3.38±0.18	4.58±0.36
42	1.75±0.21	3.33±0.15	4.37±0.17

4. Conclusions

In conclusion, this research is the first study to show that changes in serum and milk leptin in beef cows during lactation might play a role in regulation of their offspring's body weight in early life. As days postpartum were increased, the concentrations of leptin in serum and milk were decreased. There was a strong positive relation between serum and milk leptin. Both serum and milk leptin had a strong negative relation with calf body weight. Moreover, there were also no relations noted among calf serum leptin, calf body weight, cow serum leptin, and cow milk leptin. However, many bioactive hormones and milk compositions could be involved in the complex system governing calf growth. Therefore, further studies are needed to clarify the role of other hormones involved in regulation of growth and development, such as growth hormone and ghrelin.

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