Correlation between blood and milk serum leptin in goats and growth of their offspring

N. C. Whitley†, E. L. Mcfadin-Buff*, S. A. Harley† and

D. H. Keisler*

†Department of Agriculture, University of Maryland Eastern Shore,
Princess Anne, MD 21853
* University of Missouri, Columbia, MO 65211

ABSTRACT: Secreted from both fat and mammary gland tissue, leptin is a hormone that has been found in the milk of a variety of species and is positively correlated with animal body condition. It has been hypothesized that milk leptin ingested by offspring early in the postnatal period may influence growth and development of the offspring. However, it has not been determined if leptin is found in goat milk and if milk leptin levels are correlated with the growth of kids. Therefore, the objectives were to determine if goat milk serum contains leptin and to investigate possible correlations of milk and serum leptin in does and subsequent growth of their offspring. To conduct the study, Boer and Boer crossbred meat type does were used in two Experiments (Exp). Blood samples (via jugular venipuncture) and milk samples were collected within 2 hours of kidding (= d 0) from 20 (Exp 1; conducted in the Spring) or 22 does (Exp 2; conducted in the Fall) and were collected again on days 0.5, 1, 3, 5, 7, 14, 21, 28, 35, 42, 49, and 56 (Exp 1) or days 0.5, 1, 2, 3, 4, 5, 6, 7, 14, and 21 (Exp 2). Body weights of kids were taken at d 0, and body weights of both kids and does were measured weekly beginning on d 7 (kids, Exp 1) or d 21 (does, Exp 1) or on days 0.5, 1, 2, 3, 4, 5, 6, 7, 14, and 21 (Exp 2). Blood and milk serum leptin concentrations were determined using radioimmunoassay. Leptin was detected in milk and averaged 4.4 ± .32 ng/ml (Exp 1) and 18.1 ± .95 ng/ml (Exp 2) on d 0, decreasing to 1.0 ± .31 ng/ml on d 56 (Exp 1) and 2.9 ± 0.2 ng/ml on d 21 (Exp 2) with day postpartum and doe milk serum leptin negatively correlated (r^2 = -.07; $p < 0.0001$ for Exp 1 and r^2 = -.21, $p < 0.0001$ for Exp 2). Days postpartum and blood serum leptin were positively correlated in Exp 1 (r^2 = .02; $p < 0.02$) only. As expected, positive correlations ($p < 0.01$) were found between leptin and doe body condition (r^2 = .17, Exp 1 and r^2 = .03, Exp 2) and doe body weight (r^2 = .20, Exp 1 and r^2 = .06, Exp 2). In conclusion, leptin was present in milk and blood serum of does and was correlated with doe body weight and body condition but not with kid body weight.

Introduction

Leptin is a 16 kDa protein secreted from adipocytes (Houseknecht et al., 1998) that is involved in the regulation of food intake, energy expenditure, body temperature regulation, and whole body balance in rodents and humans. Leptin is produced by fat cells but has also been found in placenta (Hassink et al., 1997; Hoggard et al., 1997a, 2000; Ashworth et al., 2000) where it is passed through to the fetus during its transition to neonate (Matsuda et al., 1999). In addition, leptin has been found in milk of humans as well as other animals. Therefore, although not presently known, it is highly likely that leptin is also found in goat milk and may be related to body weight and/or body condition score of the female. However, the possible effects of milk leptin on growth of goat kids are unknown. Therefore, the objectives of two experiments (EXP) were to 1) determine the relationship between doe milk serum leptin and doe blood serum leptin levels in postpartum does and 2) determine possible correlations of doe blood and milk serum leptin with doe body condition score (BCS), doe body weight (BW) body weights of their offspring.

Materials and Methods

Animals

Mixed-parity Boer and Boer crossbred meat type does approximately 2.8 ± .2 years of age were placed in a 2.5 ha dry lot area at least 4 days before the first expected kidding date and were fed ad libitum grass hay and approximately .45 kg/doe/day of a 16% CP corn-soybean meal diet. After kidding, does and kids were placed in individual 1.5 x 1.5 m pens in a barn on concrete floors with straw bedding for the duration of the study. Animals were allowed ad libitum access to water and were presented with grass hay and a 16% CP corn-soybean meal diet twice daily at levels to attempt to maintain at least a 2.5 body condition score (on a scale of 1-5

with 1 being very thin and 5 being obese). Within 2 hours after parturition (= d 0), kids were weighed and ear tagged and sex was recorded and doe blood (via jugular venipuncture) and milk samples were collected and stored at –20^{o} C until further processing. All animal-related procedures were approved by the UMES Animal Care and Use Committee.

Twenty does and their offspring (approximately 1.9 ± .15 kids per doe) were used in Exp 1 in the Spring of the year. After d 0 samples were taken, blood and milk samples were again taken at 12 (d 0.5) and 24 h (d 1) after kidding, as well as on d 3, 5, 7, and 14. Beginning on d 21, blood samples, milk samples, doe BCS and doe BW were taken weekly (d 21, 28, 35, 42, 49, and 56). In addition, kid BW was recorded weekly from d 7 until the end of the study at d 56. Twenty-two does and their offspring were used in Exp 2 in the Fall of the year. Based on results from Exp 1, more intensive sampling was conducted in the early postpartum period and the duration of sampling shortened to 21 days. After d 0 samples were taken, blood and milk samples and kid body weights were taken again at d 0.5, 1, 2, 3, 4, 5, 6, 7, 14 and 21.

After collection, blood was stored at 4°C and allowed to clot overnight. Serum was collected after centrifugation at 1,200 x g for 20 minutes and stored at -20°C. Milk samples were ultracentrifuged at 45,000 X g at 5° C for 1 hour and the clear supernate was extracted and stored at -20°C. Blood and milk serum leptin concentrations were measured using radioimmunoassy as previously described by Devlaud and coworkers (2000).

Statistical Analysis

Pearson product moment correlations between the variables doe milk serum leptin, doe blood serum leptin, doe body weight, doe body condition score, days postpartum and kid weights were calculated using the CORR procedure of SAS. This procedure produces single bivariate correlation coefficients and related significance tests, automatically generating the Pearson r

(Dilorio and Hardy, 1996). Partial correlations were also obtained using day, number of nursing kids, and/or doe body condition score as the controlling variable when applicable (SAS Procedures Guide, p. 277; Cary, NC; 1999). A partial correlation measures the strength of a relationship between two variables while controlling the effect of one or more additional variables (SAS Procedures Guide, p. 291; Cary, NC; 1999). The MIXED procedure of SAS was used to calculate the effect of day on milk and serum leptin concentrations as well as doe body condition score with doe considered the repeated variable and AR(1) specified. The GLM procedure of SAS was also used with the MANOVA option to obtain possible effects of day as well as partial correlation coeffcients with results similar to those obtained with MIXED and CORR procedues with day as the controlling variable.

Results

Experiment 1

As days postpartum increased, levels of doe milk serum leptin decreased ($P < 0.001$; Figure 1) with a positive correlation was noted between days postpartum and milk serum leptin ($r^2 = -.07$; $P < .0001$). In contrast, doe blood serum leptin tended to increase ($p < .10$) with days postpartum with a slight positive correlation between days postpartum and serum leptin ($r^2 = .02$; $p < .02$; Figure 1). For other data, partial correlation coefficients using day and number of suckling kids were used as controlling variables to account for those effects and as expected, serum blood leptin was positively correlated with doe BCS ($r^2 = .10$; $p < .002$) and doe BW ($r^2 = .21$; $p < .0001$). Without using controlling variables, the initial correlations for serum blood leptin were $r^2 = .17$ ($p < .0001$) with doe BCS and $r^2 = .20$ ($p < .0001$) with doe BW, and a negative relationship existed between doe milk serum leptin concentrations and kid weight ($r^2 = .06$; $p < 0.0009$). However, the association between doe mik serum leptin and kid BW was

accounted by day and number of suckling kids and was no longer apparent when using those factors as controlling variables.

Experiment 2

As days postpartum increased, levels of milk serum leptin decreased ($p < .0001$) with a negative correlation noted between days postpartum and milk serum leptin ($r^2 = -0.21$; $p < 0.0001$; Figure 2) while no relationship was found between blood serum leptin and days postpartum ($p > .10$; Figure 2). As expected, doe blood serum leptin was positively correlated ($p < .0005$) with doe BCS ($r^2 = .03$) and doe BW ($r^2 = .06$). Without controlling for day or number of nursing kids the correlations with blood serum leptin were $r^2 = .01$ with doe BCS ($p < .09$) and $r^2 = .07$ with doe BW ($p < .0002$). An initial correlation existed between milk serum leptin concentrations and kid body weight ($r^2 = -0.10$, $p < 0.0001$), however, as in Experiment 1, when day and number of nursing kids was accounted for, the relationship no longer existed.

Discussion

Leptin was found in goat milk serum, as noted with other species, such as humans, mice, pigs, sheep and horses (Houseknecht et al., 1997; Aoki et al., 1999; Estienne et al., 2000; McFadin-Buff et al., 2001; Salimei et al., 2002). Leptin mRNA has also been found in mammary tissue of humans, rats and sheep (Smith-Kirwin et al., 1998; Aoki et al., 1999; Laud et al., 1999) and is regulated in bovine mammary epithelial cells by growth factors known to alter mammary function and nutrient partitioning (Smith and Sheffield, 2002).

As days postpartum increased in both studies, the level of leptin in doe milk serum decreased, with the greatest milk serum leptin levels occurring in colostral (d 0) milk samples. These results are similar to those found in sheep (McFadin-Buff et al., 2001), mares (Salimei et

al., 2002) and in pigs (Estienne et al., 2000; in skim milk) and has been attributed to perhaps a pooling of leptin in the udder prior to parturition (McFadin-Buff et al., 2001). The general protein profile in milk follows the same pattern (Fuertes et al., 1998), indicating that milk leptin is probably not distinctively or uniquely modulated. However, the peak in milk leptin occurs when neonates are best able to absorb large proteins through the gut, and elevated serum leptin has been found in neonatal suckled (Casabiell et al., 1997) compared to unsuckled rat pups (Dessolin et al., 1997) and neonatal pigs treated orally with leptin (Whitley et al., 2001) or pigs suckled versus being fed milk replacer (Weiler et al., 2002). Therefore, leptin may play a role in development of the neonate, though that possible influence is is still not understood.

Initial theories of possible milk leptin influence on the neonate were through feed intake and/or growth potential regulation, but those theories have not been fully supported by research. Exogenous leptin did not influence food intake in neonatal mice 7-10 days of age (Mistry et al., 1999) and leptin in breast milk did not influence satiation at the end of suckling in human infants (Ucar et al., 2000). However, recent reports have noted possible delayed effects of early leptin influence. In 15-day old pigs treated earlier in life (5 day suckled, unsuckled, dexamethasone or placebo), plasma leptin was predicitive of bone and fat mass after accounting for body size and treatment effects and suckled pigs exhibited greater initial plasma leptin concentrations than those fed milk replacer (Weiler et al., 2002). In humans, breast milk intake was significantly associated with lower leptin concentrations relative to fat mass in adolescence (Singhal et al., 2002) even after adjustment for age, sex, Tanner stage, social class and fat mass. However, no apparent relationships between dam milk leptin and offspring growth were noted for goat kids in the present study. Although milk leptin was initally negatively correlated with kid body weight, the two appear to be independent of each other because after accounting for other major

influences on kid weight (day and number of kids), the two were no longer correlated. Therefore, present study upholds the current lack of convincing evidence for leptin as a direct modulator of neonatal growth. However, milk leptin could be important for indirect developmental effects, kid survival or other factors not measured in this study.

A more probable role of leptin in the neonate may be in thermoregulation. Leptin was shown to alter sympathetically mediated themoregulatory thermogenesis to augment cold defense abilities in rat pups (Stehling et al., 1997). Also, in neonatal rats, the ability of leptin to accelerate metabolic rates was acquired early in life (by day 17) and was theorized to promote survival of neonates (Mistry et al., 1999). More recently, in neonatal lambs, Mostyn et al. (2002) reported that leptin administration prevented the normal decline in colonic temperature over the first few hours and days after birth. In addition, at seven days of age, colonic temperature was strongly correlated with UCP1 mRNA abundance and thermogenic potential in leptin-treated lambs (Mostyn et al., 2002). Uncoupling proteins are characteristic of brown adipose tissue, which is important for thermogenesis in neonates of many mammalian species. Like lambs and rats, neontal goats also possess brown adipose fat at birth that markedly decreases over the first few weeks of life (Trayhurn et al., 1993). Concidentally, although not compared statistically, milk leptin was higher in colostrum of does kidding in the Fall compared to those kidding in the Spring, indicating a possible influence of season of the year or seasonal temperature.

Doe blood serum leptin was related to body condition score and body weight in both EXP in the present study, however the relationships were much stronger in does kidding in the Spring (EXP 1) than in does kidding in the Fall from an induced out-of-season breeding regime. There are similar reports of positive correlations between circulating concentrations of leptin and body fatness (Buff et al., 2000; Delavaud et al., 2000; Maffei et al., 1995) and body weight

(Considine, 1996) in other species. The weak relationship between serum leptin and BCS in EXP 2 is consistent with the lack of BCS changes during the study (BCS = 2.77 ± .01) and is similar to results reported in ewes that maintained their body condition scores throughout lactation (McFadin-Buff et al., 2001; BCS = 2.86 ± 0.59).

Implications

Leptin is present in the milk of goats and, as with other species, concentrations are highest just after parturition and decline quickly. As expected, serum leptin was correlated with body condition score and body weight in does. However, a link between milk leptin and kid growth was not realized and further studies are needed to determine if current theories about leptin involvement in thermogenesis or neonatal development are valid.

Literature Cited

Aoki, N., M. Kawamura, and T. Matsuda. 1999. Lactation-dependent down regulation of leptin production in mouse mammary gland. Biochim Biophys Acta. 1427:298-296.

Ashworth CJ, N. Hoggard, L. Thomas, J.G. Mercer, J.M.Wallace & R.G. Lea. 2000.Placental Leptin. Reviews of Reproduction. 5:18-24

Buff, P. R., A.C. Dodds, C. D. Morrison, N. C. Whitley, E. L. McFadin-Buff, J. A. Daniel, J. Djiane, D. H. Keisler. 2001a. Leptin in horses: tissue localization and relationship between peripheral concentrations and body condition. J. Anim. Sci. (In Press).

Casabiell, X., V. Pineiro, M. Tome, R. Peino, C. Dieguez, and F. Casanueva. 1997. Presence of leptin in colostrum and/or breast milk from lactating mothers: a potential role in the regulation of neonatal food intake. J. Clin. Endocrinol. Metab. 82:4270-4273.

Delavaud C., Bocquier F., Chilliard Y., Keisler D. H., Gertler, and Kann G. 2000. Plasma leptin determination in ruminants. J. Endocrinol. 165:519-526.

Dessolin S., M. Schalling, O. Champigny, F. Lonnqvist, G. Ailhaud, C. Dani and D. Ricquier. 1997. Leptin gene is expressed in rat brown adipose tissue at birth. FASEB J. 11:382-387.

Dilorio, F. C. and K. A. Hardy, eds. 1996. Quick start to data analysis with SAS. Wadsworth Publishing Co., Belmont, CA.

Estienne, M. J., A. F. Harper, C. R. Barb, and M. J. Azain. 2000. Concentrations of leptin in serum and milk collected from lactating sows differing in body condition. Domest Anim Endocrinol 19:275-280.

Hassink SG, E. de Lancey, D.V. Sheslow, S.M. Smith-Kirwin,D.M. O'Conner, R.V. Considine, I. Opentanova, K. Dostal, L. Spear, K. Leef, M. Ash, A.R. Spitzer & V.L.Funanage.

1997. Placental leptin: an important new growth factor in intrauterine and neonatal development. Pediatrics. 100:124-129.

Hoggard N., L. Hunter, J.S. Duncan, L.M. Williams, P. Trayhurn and J.G. Mercer. 1997a. Leptin and leptin receptor mRNA and protein expression in the murine fetus and placenta. Proceedings of the National Academy of Sciences USA. 94:11073-11078.

Hoggard N., L. Hunter, R.G. Lea, P. Trayhurn and J.G. Mercer. 2000. Ontology of the expression of leptin and its receptor in the murine fetus and placenta. British Journal of Nutrition. 83:317-326.

Houseknecht, K. L., M. C. McGuire, C. P. Portocarrero, M. A. McGuire, and K. Beerman. 1997. Leptin is present in human milk and is related to maternal plasma leptin concentration and adiposity. Biochem. Biophys. Res. Commun. 240:742.

Houseknecht, K.L., C.A. Baile, R.L. Matteri, M.E. Spurlock. 1998. The Biology of Leptin: a review. J. Anim. Sci.76:1405-1420.

Laud, K., I. Gourdou, L. Bélair, D. H. Keisler, and J. Djiane. 1999. Detection and regulation of leptin receptor mRNA in ovine mammary epithelial cells during pregnancy and lactation. FEBS Letters. 463:194-198.

McFadin-Buff, E.L., C.D. Morrison, P.R. Buff, N.C. Whitley, and D.H. Keisler. 2001. Leptin levels in peri-parturient ewes and their subsequent offspring. J. Anim. Sci. 80:738-743.

Maffei, M., J. Halaas, E. Ravussin, R. E. Pratley, G. H. Lee, Y. Zhang, H. Fei, S. Kim, R. Lallone, S. Ranganathan, P. A. Kern, and J. M. Friedman. 1995. Leptin levels in human and rodent: measurements of plasma leptin and ob RNA in obese and weight-reduced subjects. Nat Med. 1:1155-1161.

Matsuda J, I. Yokota, M. Idia, T. Murakami, M. Yamada, T.Saijo, E. Naito, M. Ito, K. Shima and Y. Kuroda. 1999. Dynamic changes in serum leptin concentrations during fetal and neonatal periods. Pediatric research 45:71-75.

Mistry, A.M., A. Swick, and D. R. Romsos. 1999. Leptin alters metabolic rates before acquisition of its anorectic effect in developing neonatal mice. Am J. Physiol. 277:R742-747.

Mostyn, A., J. Bispham, S. Pearce, Y. Evens, N. Raver, D. H> Keisler, R. Webb, T. Stephenson and M. E. Symonds. 2002. Differential effects of leptin on thermoregulation and uncoupling protein abundanc ein the neonatl lamb. FASEB J. 16:1438-1440.

Owen JB.1999. Genetic aspects of body composition. Nutrition. 15:60913.

SAS 1996 SAS/SAT Software: Changes and enhancements through release 6.11. SAS Institute, Inc. Cary, NC

Salimei, E. G. Varisco and F. Rosi. 2002. Major constituents, leptin and non-protein nitrogen compounds in mares' colosturm and milk. Reprod Nutr Dev 42:65-72.

Singhal, A. I. S. Farooqi, S. O'Rahilly, T. J. Cole, M. Fewtrell and A. Lucas. 2002. Early nutrition and leptin concerntrations in later life. Am J Clin. Nutr. 75:993-999.

Smith, J. L. and L. G. Sheffield. 2002. Production and regulatio of leptin in bovine mammary epithelial cells. Domest Anim Endocrinol 22:145-154.

Smith-Kirwin, S. M., D. M. O'Connor, J. De Johnston, E. D. Lancey, S. G. Hassink, and V. L. Funanage. 1998. Leptin expression in human mammary epithelial cells and breast milk. J Clin Endocrinol Metab. 5:1810-1813.

Stehling, O., H. Doring, B. Huesslein-Hildeshein, M. Olbort and I. Schmidt. 1997. Leptin does not reduce body fat content but augments cold defense abilities in thermoneutrally reared

rat pups. Pflugers Arch 434:694-697.

Trayhurn, P. M. E. Thomas and J. S. Keith. 1993. Postnatal development of uncoupling protein, uncoupling protein mRNA and GLUT4 in adipose tissue of goats. Am. J. Physiol. 265:R676-682.

Ucar, B., B. Kirel, O. Bor, F. S. Kilic, N. Dogruel, S. D. Aydogdu and N. Tekin. 2000. Breast milk leptin concentrations in initial and terminal milk samples: relationships to maternal and infant plasma leptin concentrations, adiposity, serum glucose, insulin, lipid and lipoprotein level. J. Pediatr. Endcorinol. Metabl. 13:149-156.

Weiler, H. A., H. Kovacs, C. Murdock, J. Adolphe, and S. Fitzpatrick-Wong. 2002. Leptin predicts bone and fat mass after accounting ofr the effects of diet and glucocorticoid treatment in piglets. Exp. Biol. Med. (227:639-644.

Whitley, N. C., E. L. McFadin-Buff, P. R. Buff, and D. H. Keisler. 2001. Leptin in neonatal pigs: effects of oral versus intramuscular administration. J. Anim. Sci. 79 (Suppl 1):in press.

Yemm R. S, K. L. Hossner. 2000. Studies on the Nature of Leptin Binding Proteins in Sheep. **http://ansci.colostate.edu/ran/sheep/rsy1991**

Figure 1.

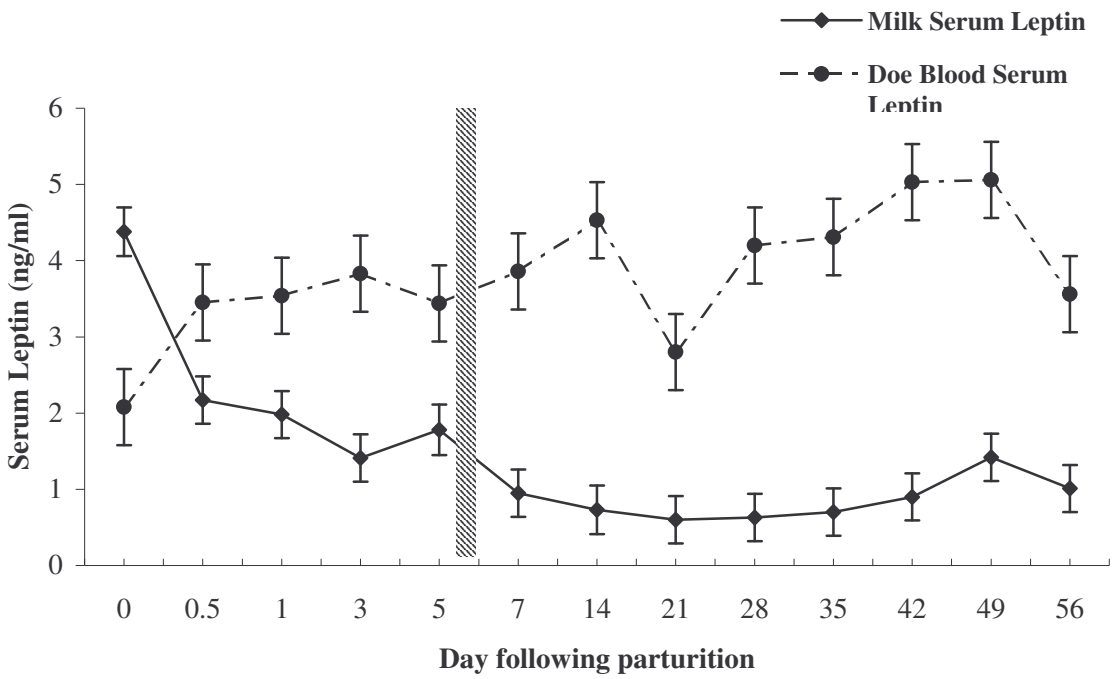

Figure 1. Milk and blood serum leptin in meat type does kidding in the Spring in samples collected within 2 hours after parturition (d 0) to 56 days postpartum.

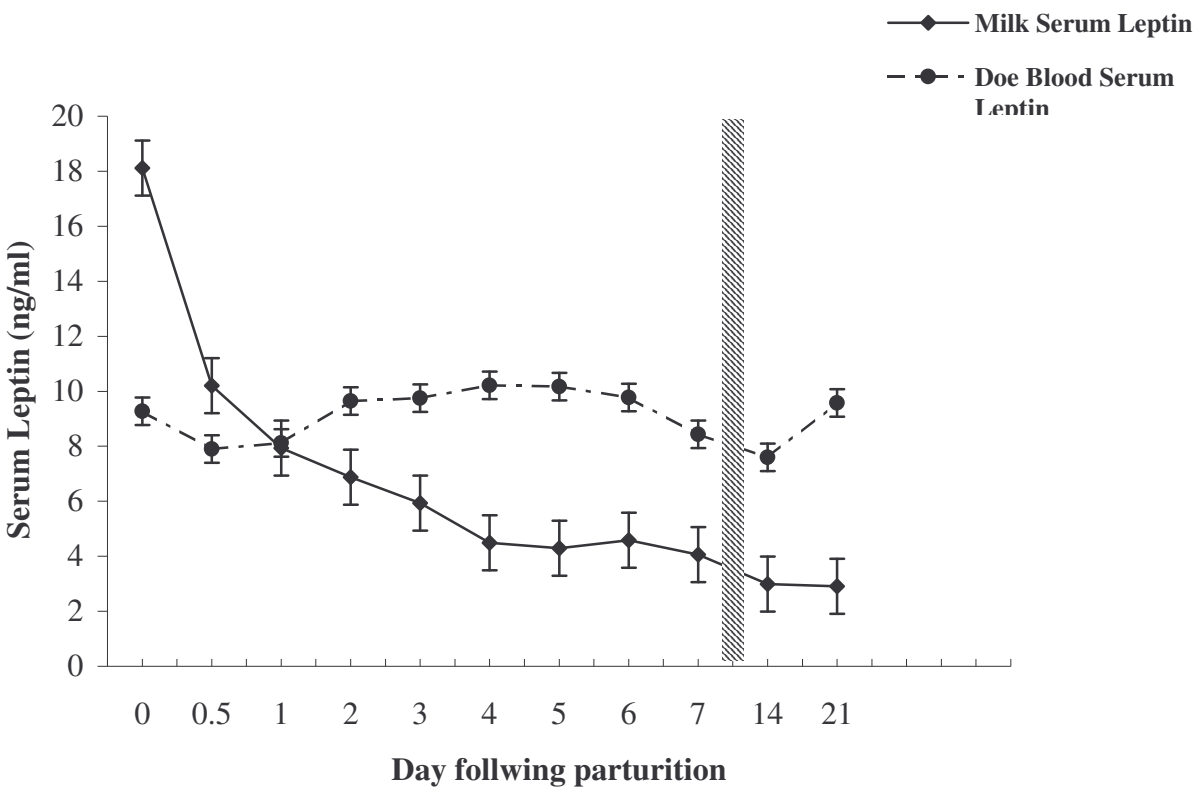

Figure 2. Milk and blood serum leptin in meat type does kidding in the Fall in samples collected within 2 hours after parturition (d 0) to 21 days postpartum.