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Plasma leptin concentration during 45 days in pre-weaning Japanese Black and Holstein calves

T. Tokuda[1] and S. Shinde[2]

[1]Laboratory of Animal Science, Faculty of Life and Environmental Science, Shimane University
Matsue-shi, 690-8504, Japan
[2]Hiroshima Prefectural Livestock Technology Research Center
Shobara-shi 727-0023, Japan

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ABSTRACT

Leptin is mainly produced in adipose tissue and acts in the hypothalamus to regulate food intake. The objective of the present study was to examine the pattern of plasma leptin concentrations of calves during 45 days after birth and the effect of breed on leptin and insulin concentrations between Japanese Black and Holstein calves. Four Japanese Black and four Holstein calves were used. Animals were fed 10% of their body weight whole milk for a week after birth, and then milk replacer and commercial formula feed for growing calf at a level to meet the nutrient requirement. Body weights were measured on the day of birth and 45 days after birth. Blood samples were collected everyday for 1-6 days after birth, 2 days interval for 6-14 days after birth and 3 days interval 14-45 days after birth to determine plasma leptin and insulin concentrations. Body weight significantly ($P < 0.05$) increased as compared to day of birth at 45 days after birth. However, plasma leptin concentration slightly decreased ($P < 0.05$) as days after birth advanced throughout the experimental period. Plasma insulin concentration decreased ($P < 0.05$) drastically 3 days after birth in both breeds. Breed of the animals influenced on plasma leptin and insulin significantly ($P < 0.05$), and high values were observed in Japanese Black than in Holstein calves. It has been suggested that the rate of body fat contents in Japanese Black cattle is naturally higher than in Holstein cattle. Probably the high levels of plasma leptin and insulin in Japanese Black calves were affected by genetically body fat characteristics of each cattle, though it was not confirmed in present study.

KEY WORDS: leptin, insulin, calves

[1] Corresponding author: e-mail: ttomo@life.shimane-u.ac.jp

INTRODUCTION

Leptin is a hormone mainly secreted from adipose tissue, though it is reported the site of leptin production is much more wide spread to the stomach, muscle, placenta faetal tissue, etc. (Andrews, 1998). Plasma leptin concentration is positively correlated with body fat mass in rodents and man (Frederich et al., 1995; Caro et al., 1996). In ruminants plasma leptin concentrations were also related with body fat (Delavaud et al., 2002), and it was affected by growing and nutritional status (Tokuda et al., 2001a, 2002). Additionally, insulin plays an important role in lipid metabolism as well as leptin. In previous study, plasma insulin concentration was increased with fattening and it was positively correlated with plasma leptin concentration in Japanese Black cattle (Tokuda et al., 2001b).

Japanese Black cattle are known as Wagyu have a unique fat deposition pattern characterized by a greater extent of marbling (Lunt et al., 1993). Additionally, Japanese Black cattle had greater carcass fat proportions and lesser carcass lean and bone proportions than Holstein cattle (Ozutsumi et al., 1984). The body skeletal muscle and fat contents are quite different between Japanese Black and Holstein cattle. Different cattle types like dairy and beef cattle probably show different development of muscle and fat tissue during postnatal growth.

In lambs, plasma leptin concentrations were determined during 45 days after birth, and the increase in plasma leptin concentration was shown just after birth (Tokuda et al., 2003). However, it has not been reported changing pattern of plasma leptin concentration and differences of breed in neonatal calves.

The objective of the present study was to examine the pattern of plasma leptin concentration of calves during 45 days after birth and the difference of plasma leptin concentration between Japanese Black calves as beef cattle and Holstein calves as dairy cattle just after birth. Additionally, plasma insulin concentration was determined, and the relationships between plasma leptin and insulin were also examined in newborn calves.

MATERIAL AND METHODS

Animals and procedures

The newborn four Japanese Black calves (three males and one female) and four female Holstein calves were used during 45 days in this trial. After the animals ingested colostrum from their dams, they were fed mature whole milk collected from different lactating cows from their dams at 10% of their body weight for a week. After that they were fed milk replacer and commercial formula feed for growing calf at a level to meet the nutrient requirements of growing calf

according to the Japanese Feeding Standards for beef cattle (Agriculture, Forestry and Fisheries Research Council Secretariat, 1995) until 45 days after birth. The nutrient compositions of feed were showed in Table 1. The animals were weighted on the day of birth and 45 days after birth.

TABLE 1

Nutrient composition of feed in fresh matter, %

Item	Milk replacer	Commercial formula feed
Dry matter	85.0	85.0
Organic matter	93.2	94.1
Crude protein	26.8	18.5
Crude fat	16.5	2.7
Crude fibre	0.03	5.1

Plasma sampling and analyses

First blood samples were collected from jugular vein after ingestion of colostrum, and then blood samples were collected just before the morning feeding at 8:30 everyday for 2-6 days after birth, 2 days interval for 6-14 days after birth and 3 days interval 14-45 days after birth. Blood samples were placed into heparinized tubes and immediately centrifuged at 1,400 × g for 15 min. Thereafter plasma samples were taken and stored at -80°C until analysis. Plasma leptin were determined using a commercial radioimmunoassay kit (Multi-species Leptin RIA kit, Linco Research, St. Charles). Human leptin was used as a standard in the assay. The intra- and inter-assay coefficients of variation were less than 6%. Plasma insulin was determined using a radioimmunoassay kit (DPC Insulin Kit, Diagnostic Products Corporation, Los Angeles, CA). The intra- and inter-assay coefficients of variation were 5.1 and 6.3%, respectively.

Statistical analysis

All values were expressed as means and standard error. All the data including the breed difference data were analysed by repeated measured analysis of variance (ANOVA). The correlation Z test was used to analyse correlations between plasma leptin and insulin concentrations. All statistical analyses were conducted using a commercially available computer program (SAS, 1999).

RESULTS AND DISCUSSION

Body weight at 45 days significantly ($P < 0.001$) increased as compared with day of birth. However, there was no significant effect of breed ($P=0.68$) and day × breed ($P=0.18$) (Figure 1).

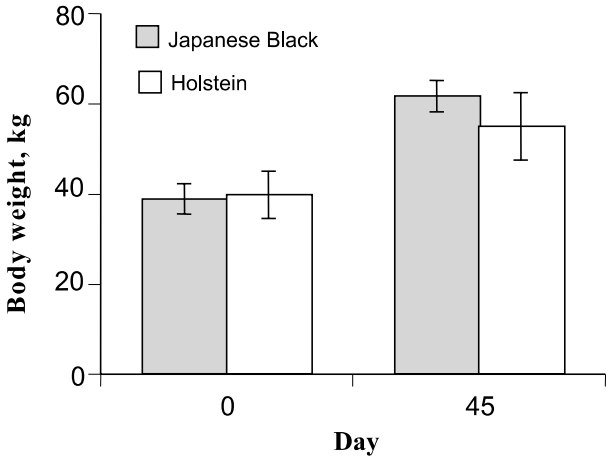

Figure 1. Body weight at just birth and 45 days after birth in Japanese Black and Holstein calves. Data are means (n=4) and s.e. For body weight: effect of time ($P < 0.001$), effect of breed ($P = 0.68$), effect of time × breed ($P = 0.18$)

The effect of day was significant ($P < 0.05$) in plasma leptin concentration. Plasma leptin concentration decreased with advance of the day throughout the experiment in spite of the increase of body weight (Figure 2). Although plasma leptin concentration in Japanese Black calves showed slightly high level at 2 days after birth, plasma leptin concentration at day of birth had high variations between animals and the increase of plasma leptin at 2 days after birth was not significant ($P > 0.05$) as compared with that at day of birth. In human, serum leptin concentration in umbilical cord blood showed high concentration, and then serum leptin concentration rapidly began to decrease with advancing the day (Matsuda et al., 1999). Placenta was reported to express leptin mRNA (Masuzaki et al., 1997), and rapid decline of circulating leptin after birth was probably due to loss of placental source of leptin. Moreover, it might reflect the dramatic changes of the hormonal and nutritional state during the perinatal period. In rats, plasma leptin concentration was undetectable at day of birth, after that it increased until approximately 1.5 ng/ml and was kept constant from 8 to 22 (weaning) days after birth (Watanobe and Schiöth, 2002). In mice, plasma leptin increased 5-10-fold in 10 days after birth and decreased to adult levels after weaning (Ahima et al., 1998). On the other hand, our previous study showed increase of plasma leptin concentration at 3 and 4 days after birth in sheep (Tokuda et al., 2003). Similarly, McFadin et al. (2002) reported that serum leptin concentration was increasing from birth of the day to 5 days after birth in sheep, and leptin concentrations in milk were greatest in the initial milk and decreased until 5 days thereafter it remained at constant level. The elevated circulating concentration of leptin after birth in sheep

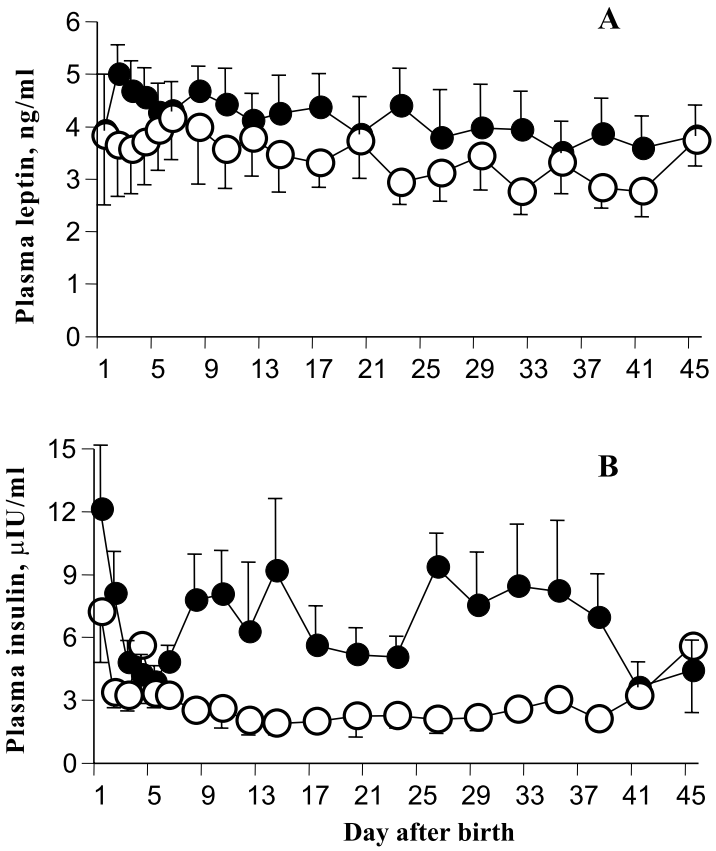

Figure 2. Changes in plasma leptin (A) and insulin (B) concentrations throughout the experiment in Japanese Black (●) and Holstein (○) calves. Blood samples were collected just before the morning feeding at 8:30 h. Data are means (n=4) and s.e.
For plasma leptin concentration: effect of time ($P < 0.01$), effect of breed ($P < 0.05$), effect of time × breed ($P = 0.64$). For plasma insulin concentration: effect of time ($P < 0.05$), effect of breed ($P < 0.05$), effect of time × breed ($P = 0.15$)

was presumably caused by high leptin concentration in milk because of transition into the circulation of neonate (Casabiell et al., 1997). Circulating hormone and metabolite could have affected also. Thus, the changing patterns of circulating leptin at neonatal period are different depending on species, though it is not clear that what factor mainly affects on changes in circulating leptin after birth.

Plasma leptin concentration in Japanese Black cattle was higher ($P<0.05$) than those in Holstein cattle (Figure 2). Ren et al. (2002) compared plasma leptin concentrations between Charolais as beef cattle and German Holstein as dairy

cattle at 18 months of age using the same assay system with present study. Contrary to present results, the plasma leptin concentration of German Holstein tended to be higher than that of Charolais. German Holstein cattle are characterized by a higher fat content as compared to Charolais cattle (Ren et al., 2002). On the other hand, Japanese Black cattle had higher carcass fat proportions than Holsteins (Ozutsumi et al., 1984). These differences between two experiments might have been occurred by the difference of meat characteristic in beef cattle, because circulating leptin concentration was affected by body fat mass in cattle (Delavaud et al., 2002). Wegner et al. (2001) have reported that plasma leptin concentrations increased in relation to the percentage of Wagyu (Japanese breed) in the cattle, in experiment using crossbred cattle with 0, 50 and 75 % Wagyu in genetic makeup. In their study, plasma leptin concentration increased with aging, and increasing rate of it with aging were higher in cattle had high genetic rate of Wagyu (Wegner et al., 2001). Therefore, the difference of plasma leptin concentration between Japanese Black and Holstein cattle could be significantly greater with aging. In fact, Higashiyama et al. (2003) showed that intramuscular lipid contents were much greater in Japanese Black cattle than in Holstein cattle and plasma leptin concentrations were also higher in Japanese Black than in Holstein cattle throughout fattening period. Probably, plasma leptin concentration have related with body fat contents since they are born, and body fat contents mainly depend on genetic factor of each breed in cattle.

In present study, three males and one female were used in Japanese Black cattle, whereas four females were used in Holstein cattle. It was shown that plasma leptin in female was higher than that in male in mature sheep and cattle (Blache et al., 2000; Ehrhardt et al., 2000; Tokuda and Yano, 2000). However, sexual difference of circulating leptin concentration was not observed in neonatal sheep (McFadin et al., 2002; Tokuda et al., 2003). In human, sexual difference was observed in adult (Rosenbaum et al., 1996) and in neonatal (Matsuda et al., 1997). The administration of testosterone caused decrease of serum leptin level in young men (Luukkaa et al., 1998), whereas oestradiol supplementation reversed the effect of ovariectomy on circulating leptin levels though serum leptin concentration decreased by ovariectomy in rats (Shimizu et al., 1997). Thus, it was suggested that sexual hormones affected remove gender difference of circulating leptin concentration in mature animal. However, there were no difference in the serum concentrations of oestradiol and testosterone did not differ between neonatal male and female (Matsuda et al., 1997). The changes in plasma leptin concentration were related to changes in body fatness and nutritional status in ruminants (Delavaud et al., 2002). Therefore, main effects on circulating leptin concentration are not sexual hormones but probably nutritional status and/or body fatness in pre-pubertal animal. Although the ratio of male and female on the experimental animals was inconsistent between breeds in present study, it was

unlikely that the effect of sex difference did affect plasma leptin concentrations in neonatal calves.

Plasma insulin concentration changed significantly ($P<0.05$), and the pattern of changes differed between two breeds (Figure 2). Plasma insulin concentration in both of breeds dramatically decreased for 3 days after birth. Similarly to present study, Schiessler et al. (2002) showed that plasma concentration of insulin in calves sucking colostrum and milk at an automatic feeding station significantly decreased from birth up to 3 days of life, though that in calves suckling their dams increased for that duration. Insulin concentration of dam's milk is highest in the first colostrum and 65 times of mature milk and it decrease with the progress of day (Blum and Hammon, 2000). When blood samples were taken from calves they had already ingested colostrum from their dams, after that calves were fed mature whole milk and milk replacer. Therefore, the high level of insulin at day 1 after birth might have been caused by ingestion of colostrum from their dams, and probably insulin in colostrum transited into the circulation of neonatal calves. After 3 days of birth, plasma insulin concentration in Holstein cattle hardly changed until the end of experiment. On the other hand, that in Japanese Black cattle fluctuated from 4 to 12 μIU/ml. Plasma insulin is partly affected by fat metabolism, and Japanese Black cattle have a unique fat deposition pattern which is characterized by a greater extent marbling (Lunt et al., 1993). This unique fat metabolism in Japanese Black calves might have affected fluctuation of insulin during neonatal period.

Moreover, plasma insulin concentration in Japanese Black cattle showed higher values ($P<0.05$) than those in Holstein cattle. Matsuzaki et al. (1997) demonstrated that plasma insulin concentration in Japanese Black cattle was higher than that in Holstein cattle, and it increased with rise of body weight. In addition, it was reported that plasma insulin concentration was positively related to carcass fatness in cattle (Trenkle and Topel, 1978). Japanese breed has a higher rate of deposition of intramuscular fat (Lunt et al., 1993) and a higher fat percentage in the carcass (Ozutsumi et al., 1984). Although both of leptin and insulin relate with lipid metabolism and change depending on amount of fat, leptin and insulin were not significantly correlated ($r=0.02$, $P=0.03$) in present study suggesting that they are not closely linked at neonate. Similarly, the relationships of plasma leptin and insulin were not obtained during 45 days after birth in sheep, though our previous study showed relationships between leptin and insulin concentration in growing lambs (Tokuda et al., 2001a) and fattening cattle (Tokuda et al., 2001b).

In summary, plasma leptin concentration during 45 days after birth slightly decreased with advance of the day, and the relationships between plasma leptin and insulin concentration were not observed. Additionally, both of hormone concentrations were higher in Japanese Black than in Holstein calves. The difference between breeds presumably was affected by genetically body fat characteristics of each breed in

plasma leptin and insulin concentration and the difference of the concentration would be clear with growing or fattening in cattle. However, the present study was not able to examine plasma leptin concentration from birth to finishing age and evaluate meat characteristics of each cattle. Long term and further detailed research are needed to clarify the meaning of leptin in cattle.

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STRESZCZENIE

Zmiany w stężeniu leptyny w osoczu krwi cieląt ras Czarna japońskia i holsztyńskiej w ciągu 45 dni po urodzeniu

Leptyna jest wytwarzana głównie w tkance tłuszczowej i w podwzgórzu i pełni rolę regulatora pobierania paszy. Celem podjętych prac było zbadanie zmian w stężeniu leptyny w osoczu krwi cieląt w ciągu 45 dni po urodzeniu oraz wpływu rasy cieląt (Czarna japońska i holsztyńska) na stężenie leptyny i insuliny. Doświadczenie przeprowadzono na 4 cielętach każdej rasy. Przez pierwszy tydzień po urodzeniu cielęta otrzymywały pełne mleko w ilości 10% masy ciała, a następnie preparat mlekozastępczy i mieszankę przemysłową dla rosnących cieląt w ilości pokrywającej zapotrzebowanie na składniki pokarmowe. Zwierzęta ważono w dniu urodzenia i w 45 dniu życia. Próby krwi pobierano codziennie od pierwszego do szóstego dnia życia, co drugi dzień od 6-14 dnia życia oraz co 3 dni od 14 do 45 dnia po urodzeniu. Masa ciała cieląt była istotnie większa ($P < 0,05$) w 45 dniu życia niż w dniu urodzenia. Stężenie leptyny w osoczu obniżało się stopniowo ($P < 0,05$) w miarę upływu dni po urodzeniu, a stężenie insuliny obniżyło się gwałtownie ($P < 0,05$) trzeciego dnia po urodzeniu u cieląt obydwóch ras, przy czym stężenie leptyny i insuliny w osoczu było wyższe ($P < 0,05$) u cieląt rasy Czarnej japońskiej niż holsztyńskiej. U bydła rasy Czarnej japońskiej udział tłuszczu w ciele jest większy niż u bydła holsztyńskiego i stąd prawdopodobnie wysoki poziom leptyny i insuliny w osoczu krwi jest warunkowany genetycznie; nie potwierdzono tego jednak w przeprowadzonych badaniach.