Plasma Leptin and Performance of Purebred and Backcrossed Hereford throughout Grazing and Feedlot Fattening

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ABSTRACT: In a herd of 24 spring-born steers, plasma leptin and performance of selected purebred (n=5) and backcrossed Hereford (n=5) were compared in a year-round summer grazing and winter feedlot fattening. Bimonthly blood collection and body weight measurement were accomplished. The plasma samples were analyzed for leptin, insulin, total cholesterol, triglyceride, NEFA and glucose. The experimental design utilized one-way ANOVA with breed as the treatment. The purebred obtained higher plasma NEFA (p<0.001) compared to backcross, regardless of seasonal feeding systems (SFS). The backcross showed gradual increase and non-responsiveness of plasma leptin to SFS. During summer grazing, attenuation of plasma leptin and sudden elevation when shifted to winter feedlot fattening were observed in purebred. Plasma leptin obtained linear relationship with body weight of purebred (r=0.53; p<0.001) and backcrossed Hereford (r=0.49; p<0.01). The purebred and backcrossed Hereford, when shifted to summer grazing, resulted to sustained and restricted daily gain, respectively. Therefore, cattle breeds of higher growth potential exhibit significant elevation of plasma leptin after 400 kg BW, when animal starts to deposit significant body fat. (Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 7 : 954-959)

Key Words: Feedlot, Grazing, Hereford, Japanese Black, Leptin

INTRODUCTION

Leptin is a 146 amino acid product of ob gene considered as a “lipostat” signal from adipose tissues that regulate feed intake and body fatness in animals. In man, obesity has been considered a genetic disease of adipose tissue (Arner, 2000) and about 40 to 50 percent of obesity is inherited (Comuzzie and Allison, 1998). Genes and environment affect the development of obesity in man or the regulation of body weight and carcass composition in animals. The environment that contribute tremendously to body fatness is focused on feeds and feeding system. The body weight susceptibility and resistance offered low and high fat diet observed in rats (Levin and Kessey, 1998) and mouse (West et al., 1992; Frederich et al., 1995) may also exist between cattle breeds. Since there is low energy retention during grazing relative to feedlot (Hata and Hidari, 2001), different metabolic responses between breeds can be determined. The strong significant relationship of leptin and body fat measures across the species such as man (Blum et al., 1997), pigs (Robert et al., 1998), sheep (Blache et al., 2000; Ehrhardt et al., 2000) and steers (Vega et al., 2004) implies that the level of leptin is an indicator of body fat condition.

Backcrossing F₁ cows (i.e. 50% Hereford plus 50% Japanese Black) to Hereford was employed to take full advantage of the heterosis of maternal trait, particularly the marbling characteristic of Japanese Black. The measurement of ADG, plasma metabolites, insulin and leptin across the year-round summer grazing and feedlot fattening period was accomplished in purebred and backcrossed Hereford of similar age. The metabolic responses and possible reason for the differences in performance may be explained by the changes in the concentration of these metabolites and hormones. Hence the objectives were to compare the performance of purebred and backcrossed Hereford, and to determine the breed’s differences in plasma leptin, insulin, glucose, NEFA, cholesterol and triglyceride raised across the year-round summer grazing and winter feedlot fattening in northern Japan.

MATERIALS AND METHODS

Herd description

Ten heads of purebred (n=5) and backcrossed (n=5) Hereford were selected in a herd composing 14 purebred and 10 backcrossed Hereford raised at the Field Science Center for Northern Biosphere, Hokkaido University Campus. All steers were spring-born, the purebred Hereford were born at the experimental station, while the ten-backcross (Hereford×Japanese Black×Hereford) were acquired from Shintoku Breeding Farm (Hokkaido, Japan). The cultural management adopted in the region is a year-round pasture grazing from late spring to late autumn (April to October), followed by winter feedlot management
Table 1. Concentrate and roughage feed composition of purebred and backcrossed (HxBxH) steers during feedlot fattening period

| Feedstuffs      | DM (%)  | CP (%)  | CF (%)  | TDN (%) |
|-----------------|---------|---------|---------|----------|
| Grass silage    | 25.0    | 12.0    | 36.0    | 78.0     |
| Corn silage     | 25.9    | 8.5     | 32.2    | 67.2     |
| Soybean meal    | 88.3    | 52.2    | 6.3     | 86.7     |
| Hay             | 79.0    | 14.4    | 68.6    | 68.1     |
| Mineral salt    |         |         |         |          |
| Free access     |         |         |         |          |

Feedlot and grazing data gathering

The purebred and backcrossed Hereford was raised as one herd (24 steers) during grazing and feedlot fattening. During feedlot, the animals were given a diet of about 1.5% DM per kg body weight, and free access to hay and water. The diet is composed of soybean oil meal, grass or corn silage and free access to mineral salts. The concentrate and roughage feed composition are shown in Table 1. The 24 cattle at pre-experimental period (i.e. -110 to 0 days) showed that the average weight of purebred was heavier than the backcrossed Hereford (data not shown). Five heads of purebred and 5 heads of backcrossed of similar body weight and age were selected in spring, the beginning of year-round data gathering and blood sampling. Every two months, body weight was measured and blood was sampled at the jugular vein from 9:00 to 11:00 am. during grazing and 8:00 to 9:00 am during feedlot before meal intake. Blood was placed in a heparinized tube, centrifuged and the aliquot plasma was stored at -40°C until analysis.

Blood analysis

The plasma samples were analyzed for leptin using multi-species leptin RIA (Linco, St. Charles, MO) as described in the protocol with recombinant bovine leptin (rbleptin) as standard. The kit utilizes guinea pig anti-human leptin. Serial dilution of bovine plasma containing leptin showed parallelism, 4.9 ng/ml sensitivity, cross reactivity (11.22%) and 97.8% recovery of 41.9 ng/ml rbleptin in bovine plasma (Vega et al., 2002). Plasma glucose, NEFA, triglyceride, total cholesterol and insulin were analyzed in plasma using Glucose C-II kit, NEFA C kit, Triglyceride E-Test, Cholesterol E-Test (WAKO Chemicals, Japan) and ELISA kit (Mercodia AB, Sweden), respectively.

Statistical analysis

Statistical analysis utilizes one-way ANOVA with breed (purebred versus backcross) as the treatment. The declared significant level was within 5% level (p<0.05). Comparison between two breeds at various parameters such as body weight, ADG (kg per day), plasma glucose, NEFA, total cholesterol, triglyceride, leptin and insulin was performed using t-test (LSD). Linear regression analysis was performed between plasma leptin and body weight, while correlation analysis was used between plasma leptin and insulin of both purebred and backcrossed Hereford. The GLM (General Linear Model) of the SAS system statistical software was utilized for simple linear regression and correlation analysis (SAS 1998).

RESULTS

Body weight and ADG

The body weight and ADG of selected purebred was the same as backcross from -110 to 0 days (Figure 1). Five
heads of purebred and five heads of backcross were selected based on similar age and similar body weight from the time of acquisition (7 months old) until the entry to pasture area (14 months old). Summer grazing covers the period from May 8 to October 20 followed by feedlot fattening that spread from October 21 to April 17 of the following year. Since the average body weight of all backcross was smaller than purebred, the purebred selected in this experiment were smaller than their average herd size. The average daily gain of 10 days pasture grazing showed that the backcross is more susceptible to body weight reduction when shifted to lower density diet compared to purebred. Likewise, the backcross showed lower pasture performance and slow compensatory gain during winter feedlot. The low ADG of backcross (-2.0 kg/d) for two days on pasture indicates inability to consume grasses and forages which is noticeable for backcrossed and Japanese Black cattle in Hokkaido. The body weight shown in Figure 1 clearly indicates that the backcross will attain their slaughter weight later than the purebred Hereford steers.

### Table 2. Means of plasma metabolite concentration between purebred (n=5) and backcrossed Hereford (H×JB×H; n=5) with seasonal feedings system

| Plasma metabolites       | Purebred (P) | Backcross (B) |
|--------------------------|--------------|--------------|
|                          | Pasture (n=5) | Feedlot (n=5) | Average (Mean±SEM) | Pasture (n=5) | Feedlot (n=5) | Average (Mean±SEM) |
| Total cholesterol (mg/100 ml) | 80.7 ± 4.0 | 53.7 | 65.3 ± 4.0 | 82.3 | 51.1 | 64.5 ± 4.0 |
| Triglyceride (mg/100 ml) | 15.2 ± 0.6 | 7.0 | 10.5 ± 0.6 | 13.9 | 5.5 | 9.9 ± 1.1 |
| NEFA (µEq/l) | 130.0 ± 10.9 | 92.4 | 108.5 ± 10.9 | 89.5 | 56.5 | 70.1 ± 10.8 |
| Glucose (mg/100 ml) | 68.8 ± 1.3 | 77.4 | 73.7 ± 1.3 | 70.0 | 77.0 | 74.0 ± 1.3 |

In rows, means with different letters (a,b) declare significant differences (p<0.001).

Plasma metabolites

Plasma cholesterol, triglyceride and NEFA of purebred and backcross during summer grazing and winter feedlot management systems are shown in Table 2. Plasma cholesterol was not significantly different between two breeds exposed to grazing and feedlot management systems. However, comparing summer grazing and winter feedlot, the former resulted to higher plasma cholesterol, triglyceride and NEFA. As expected plasma glucose was significantly higher (p<0.001) when animals were on feedlot compared pasture, because of the effect of concentrate feed. Plasma NEFA was significantly higher in purebred compared to backcross (p<0.001) regardless of seasonal feeding systems.

### Plasma insulin and leptin

Figure 2 shows the changes in plasma leptin and insulin across the pasture and feedlot fattening period of purebred and backcross Hereford. There was no breed-related change in plasma insulin, however summer grazing obtained lower concentration compared to winter feedlot. Plasma leptin of purebred was attenuated during pasture feeding, and then elevated suddenly to significantly higher level compared backcross (p<0.01) during feedlot fattening. The backcross showed gradual plasma leptin elevation from 0 to 335 days of fattening, supporting the age-related increase reported previously (Vega et al., 2002). Figure 3, also showed the significant linear relationship of plasma leptin and body weight of purebred (r=0.53, p<0.001) and backcross (r=0.49, p<0.01). Plasma leptin and insulin were not significantly
related across the year-round fattening period of purebred (r=0.01; p=0.658) and backcrossed Hereford (r=0.001; p=0.622). The non-significant correlation of plasma insulin and leptin maybe due to limited amount and duration of concentrate feeding.

**DISCUSSION**

This experiment was conducted to understand the differences in the performance of purebred and backcrossed Hereford steers raised under two seasonal feeding systems (SFS), i.e. (1) spring to summer pasture grazing, and (2) autumn to winter feedlot fattening, culturally practiced in northern Japan. On the assumption that possible performance differences of purebred and backcrossed Hereford, the breed-related differences of plasma leptin, insulin and some related metabolite concentrations were investigated across the seasonal feeding system. The sources of variation between the two SFS employed were, (1) environmental temperature, (2) animal activity, (3) provision of concentrate feed and (4) age of the animals. Hence, comparison between SFS is complicated to evaluate. In northern Japan, the year-round system of summer grazing and winter feedlot fattening were the most appropriate management system, thus, other alternative systems were not considered. Therefore this study was accomplished primarily to compare purebred and backcrossed Hereford (25% JB) across the two feeding systems.

Hata and Hidari (2001) substantiated the low energy retention concept of grazing cattle, and this was supported by relatively higher plasma NEFA and lower plasma glucose and insulin during summer grazing compared to winter feedlot fattening in this study. Plasma NEFA obtained wider differences between grazing and control (feedlot), and the comparison of herbage and control indicated possible added effect of animal activity and herbage feed on plasma NEFA elevation (Hata and Hidari, 2001). The relatively higher plasma glucose with winter feedlot fattening was likely the effect of concentrate feed offered, because its increase in ruminant diet also increases propionate, a known glucose precursor in the blood. The plasma glucose values we obtained were lower than those of feedlot steers reported by Matsuzaki et al. (1997), which can be attributed to lower temperature during winter in our study. This lower plasma glucose in winter may be supported by parallel results in steers that cold acclimatization increased resting metabolic rates in ewes (Christopherson, 1976), implying greater maintenance energy requirement during winter.

Plasma cholesterol, triglyceride and NEFA were relatively higher in summer pasture compared to winter feedlot fattening. The total cholesterol we obtained was similar to crossbred Hereford reported by O’Kelly (1972) and in Japanese Black reported by Matsuzaki et al. (1997). The lower critical environmental temperature for feedlot finishing cattle was between -30 and -40°C according to Young (1981) which indicates high survival tolerance of feedlot finishing cattle in cold winter season. The thermal comfort zone of fattening cattle is 18°C as manifested by high gain to feed ratio (Curtis, 1983). Disregarding the effect of health condition and productivity in cows, the total cholesterol was not different between summer and cold season (Kweon et al., 1986). Level of nutrition on the other hand, could be the reason for higher elevation of plasma total cholesterol, triglyceride and NEFA during summer grazing. DiMarco et al. (1981) supported the changes of these metabolites with feeding condition, showing that plasma cholesterol, NEFA and glycerol were significantly elevated during fasting and declines with refeeding periods in 670 kg steers. Briefly, the higher concentrations of plasma triglyceride and NEFA during summer grazing relative to winter feedlot reflects lower plane of nutrition during grazing.

Plasma insulin was lower during summer grazing relative to winter feedlot, and similar between breeds. The noticeable decline in plasma insulin concentration with prolonged summer grazing may be attributed to the declining productivity of pasture grass with the period of summer grazing. Our plasma insulin level during summer grazing and winter feedlot was higher than those reported by Hata and Hidari (2001) but lower than those reported by
Matsuzaki et al. (1997). The seasonal feeding systems most likely influenced the significant differences in levels of plasma insulin. Verde and Trenkle (1987) reported higher plasma insulin in large frame compared to small frame steers with almost 100 kg difference. In our experiment the body weight difference between breeds was about 50 kg, which may not be sufficient to effect significant influence on concentrate feed intake as reflected by comparable glucose concentration. Higher plasma insulin during winter feedlot relative to summer grazing indicates the response of animal with the provision of concentrate feed. The herbage-fed steers resulted to significantly lower plasma insulin compared to control (Hata and Hidari, 2001), this finding supports the influence of concentrate feed on plasma insulin elevation.

The age-related increase in plasma leptin with the period of fattening is consistent in both breeds and those reported by Vega et al. (2002). During summer grazing the level of plasma leptin between purebred and backcrossed was not significantly different, except during feedlot fattening where significantly higher leptin concentration in purebred compared to backcross was observed. Plasma leptin is a good indicator of body lipid contents in mouse (Frederich et al., 1995) and significantly related to body fat measures of human (Blum et al., 1997), pigs (Roberts et al., 1998), sheep (Blache et al., 2000; Ehrhardt et al., 2000) and steers (Vega, 2004). The seemingly elevated and attenuated plasma leptin of backcross and purebred respectively, maybe attributed to breed-related differences in fat metabolism during grazing, which needs further study. On the other hand, the susceptibility to decrease in weight gain of backcross could be attributed to its eating behavior, hence comparison of feed intake during grazing of purebred and backcross maybe important to consider as a follow-up study. The persistence of purebred in sustaining higher ADG than backcross from summer grazing towards the middle of winter feedlot fattening period shows its superior breed potential. Compared to the breed of lower growth potential, significant elevation of plasma leptin is manifested after 400 kg body weight of cattle with higher growth potential (i.e. purebred).

Results of the body weight, ADG, plasma NEFA and leptin in a year-round SFS revealed faster growth rate and better energetic efficiency of purebred compared to backcrossed Hereford during summer grazing. Old and Garrett (1987) obtained almost similar report that Hereford used feed energy more efficiently compared to Charolais. During summer grazing, purebred having sustained ADG, significantly higher plasma NEFA and lowered tendency of plasma leptin may reflect greater lipolysis and fat mobilization. On the other hand, backcross having significantly lower ADG, lower plasma NEFA and elevated tendency of plasma leptin during summer grazing may indicate lesser fat mobilization of backcross. The shift to winter feedlot of purebred resulting to immediate elevation in plasma leptin coupled with the decline in plasma NEFA may reflect body fat deposition.

**IMPLICATIONS**

Grazing resulted to higher plasma triglyceride and NEFA, and lower plasma glucose and insulin compared to feedlot fattening. These conditions are indicative of lower plane of nutrition during grazing relative to feedlot fattening. During feedlot pre-fattening period, backcrossed Hereford, possessing 25% Japanese Black genes attained similar body weight gain with purebred. The susceptibility of backcross to decrease in body weight gain when shifted to summer grazing, contrary to the purebred’s sustained body weight gain regardless of feeding systems employed, reflected an attenuated tendency of plasma leptin and elevated plasma NEFA during grazing of purebred which are suggestive of fat mobilization and their possible role in animals’ energetic efficiency. It is concluded that breeds with higher growth potential under the condition of sufficient concentrate feed exhibited significant plasma leptin elevation after attaining 400 kg body weight, i.e. when animals starts to deposit significant body fat.

**REFERENCES**

Arner, P. 2000. Obesity-a genetic disease of adipose tissue? Br. J. Nutr. 83:S9-S16.
Blache, D., R. L. Tellam, L. M. Chagas, M. A. Blackberry, P. E. Vercoe and G. B. Martin. 2000. Level of nutrition affects leptin concentrations in plasma and cerebrospinal fluid in sheep. J. Environ. 165:625-637.
Blum, W. F., P. Englaro, S. Hanitsch, A. Juul, N. T. Hertel, J. Muller, N. E. Skakkebaek, M. L. Heiman, M. Bierkett, A. M. Attanasio, W. Keiss and W. Rascher. 1997. Plasma leptin levels in healthy children and adolescent: dependence on body mass index, body fat mass, gender pubertal stage and testosterone. J. Clin. Endocrinol. Metab. 82:2904-2910.
Christopherson, R. J. 1976. Effect of prolonged cold and outdoors winter environment on apparent digestibility in sheep and cattle. Can. J. Anim. Sci. 56:201-212.
Comizzie, A. G and D. B. Allison. 1998. The search for human obesity genes. Science 280:1374-1377.
Curtis, S. E. 1983. Thermal-environmental requirements. In: Environmental Management in Animal Agriculture (Ed. G. I. Christison, K. W. Kelley and W. O. Wilson) Iowa State University Press, Ames, Iowa, pp. 127-133.
Dimarco, N. M., D. C. Beitz and G. B. Whitehurst. 1981. Effect of fasting on free fatty acid, glycerol and cholesterol concentrations in blood plasma and lipoprotein lipase activity in adipose tissue of cattle. J. Anim. Sci. 52:75-82.
Ehrhardt, R. A., R. M. Slepetis, J. Siegal-Willot, M. E. Van Amburgh, A. W. Bell and Y. R. Boisclair. 2000. Development of specific radioimmunoassay to measure physiological
changes of circulating leptin in cattle and sheep. J. Endocrinol. 166:519-528.
Frederich, R. C., A. Hamann, S. Anderson, B. Lollman, B. B. Lowell and J. S. Flier. 1995. Leptin levels reflect body lipid in mice: evidence for diet-induced resistance to leptin. Nature Medicine 1:1311-1314.
Hata, H and H. Hidari. 2001. Characteristics in deposition of body components and endocrine system in grazing steers. Proceedings of Japanese Society of Animal Nutrition and Metabolism 45:75-91.
Kweon, O. K., H. Ono, K. Osasa, M. Onda, K. Oboshi, H. Uchisugi, S. Kurosawa, H. Yamashita and H. Kanagawa. 1986. Factors affecting serum total cholesterol level of lactating Holstein cows. Jpn. J. Vet. Sci. 48:481-486.
Levin, B. E. and R. E. Keesey. 1998. Defense of differing body weight set points in diet-induced obese and resistant rats. Am. J. Physiol. 43:R412-R419.
Matsuzaki, M., S. Takizawa and M. Ogawa. 1997. Plasma leptin, metabolite concentration and carcass characteristic of Japanese Black, Japanese Brown and Holstein steer. J. Anim. Sci. 75:3287-3293.
Old, C. A. and W. N. Garret. 1987. Effects of energy intake on energetic efficiency and body composition of beef steers differing in size at maturity. J. Anim. Sci. 65:1371-1380.
O’Kelly, J. C. 1972. Plasma lipid changes in genetically different types of cattle during hyperthermia. Comp. Biochem. Physiol. 44A:313-320.
Robert, C., M. Palin, N. Coulombe, C. Roberge, F. G. Silverside, B. F. Benkel, R. M. Mckay and G. Pelletier. 1998. Backfat in pigs is positively associated with leptin mRNA levels. Can. J. Anim. Sci. 78:473-482.
Vega, R. A., H. G. Lee, N. Matsunaga, H. Kuwayama and H. Hidari. 2002. Age-related changes in plasma leptin from early growing to late finishing stages of castrated Holstein steers: utilizing multi-species leptin RIA. Asian-Aust. J. Anim. Sci. 15(5):725-731.
Vega, R.A., H. Hidari, H. Kuwayama, M. Suzuki and D. D. Manalo. 2004. The relationship of plasma leptin, backfat thickness and TDN intake across finishing stage of Holstein steers. Asian-Aust. J. Anim. Sci. 17(3):330-336.
Verde, L. S. and A. Trenkle. 1987. Concentrations of hormones in plasma from cattle with different growth potentials. J. Anim. Sci. 64(2):426-432.
West, D. B., C. N. Boozer, D. L. Moody and R. L. Atkinson. 1992. Dietary obesity in nine inbred mouse strain. Am. J. Physiol. 31:R1025-R1032.
Young, B. A. 1981. Cold stress as it affects animal production. J. Anim. Sci. 52:154-163.