The Relationships of Plasma Leptin, Backfat Thickness and TDN Intake across Finishing Stage of Holstein Steers

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ABSTRACT : Six 16 months old Holstein steers were offered *ad libitum* feed for 7 months, to determine the (1) relationships of backfat thickness (BFT) to plasma leptin, and insulin; and (2) associations of TDN intake/kg body weight (BW) to plasma leptin, BFT and insulin. Feed intake, body weight and BFT were measured on selected monthly ages from day 1 to 8, day 1 and 8, and day 8, respectively. Blood was sampled on day 8 and the plasma was analyzed for leptin, insulin, glucose, NEFA, total cholesterol and triglyceride. Body weight and BFT increased, while TDN intake per kg BW decreased from 16 to 23 months old. Plasma leptin increased and mimicked the level of insulin, resulting to significant correlation ($r=0.54$; $p<0.002$). TDN intake was negatively related to plasma leptin ($r=0.49$; $p<0.004$), insulin ($r=0.41$; $p<0.02$) and BFT at 12 to 13th rib ($r=0.48$; $p<0.005$). Backfat thickness at 12 to 13th rib was positively related to plasma leptin ($r=0.45$; $p<0.01$). Negative associations of TDN intake with plasma leptin and BFT during finishing period suggest long-term involvement of adipose tissues in the feed intake regulation of steers fed high concentrate diet. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 3 : 330-336)***

Key Words : Leptin, TDN Intake, Backfat Thickness, Holstein Steers

INTRODUCTION

Regulation of feed intake and body fat is of utmost importance for humans because of obesity related diseases. In domestic animals, understanding the regulation of feed intake and body fat accumulation may be economically rewarding. There are several evidences on the involvement of fat tissue in regulating feed intake and body weight in experimental animals. Kennedy reported in 1953 the involvement of fat depot in feed intake of rats, the genetically obese-diabetic mice parabiotically joined with normal mice resulted to loss in weight, hypoglycemia and death of normal partner due to starvation (Coleman and Hummel, 1969), and injection of acid-ethanol extracts of rat adipose tissue resulted in feed inhibition of mice (Goodner and Goodner, 1996). The cloning of mouse obese gene set a landmark in clarifying the adipose derived inhibitor of feed intake (Zang et al., 1994). Thus, leptin has been considered as "lipostat", a signal from adipose tissue that control body fat through feed intake and energy metabolism.

In lambs, central infusion of leptin in well-fed ewes caused reduction on feed intake, but not in food deprived ewes (Morrison et al., 2001). In sheep there was no evidence of circadian rhythm of plasma leptin (Marie et al., 2001), suggesting that the short-term meal intake may not be regulated by plasma leptin. In large animals, evidence of the regulation of feed intake, body weight or body fat by leptin remains unclear. Plasma leptin was positively related to body fat content in male and female subjects (Moller et al., 1998), backfat thickness of sheep (Blache et al., 2000a) and fat score in sheep (Ehrhardt et al., 2000). Subcutaneous fat was highly related to plasma leptin in human (Takahashi et al., 1996) and pigs (Robert et al., 1998). Clearly, leptin has become a good indicator of body fat, but whether it regulates feed intake and body fat remain controversial.

There has been no better alternative in measuring body fat directly in living animals but through ultrasonic technique of backfat thickness (BFT). It was reported that the ultrasound technique accurately measures BFT in cattle (Brethour, 1992; Houghton and Turlington, 1992). Considering this, we performed ultrasonic scanning on seven months-old cross-sectional study of finishing Holstein steers fed with *ad libitum* and high concentrate diet to monitor the changes in TDN intake/kg BW at fattening period and determine its relationship to plasma leptin and BFT.

MATERIALS AND METHODS

Care and management of animals

Six castrated Holstein steers were housed in a pen with individual electronic headgates. For the period of one month (15 months-old), the steers underwent adaptation and all animals readily consumed 2.0 to 2.3% concentrate feed per kg body weight and 1.8 kg roughage feed every day. At the start of adaptation period, the level of feeding was maintained on the basis of *ad libitum* feeding (i.e. more than 10% orts) and this was carried through the end of the experiment. Feed intake was measured at selected monthly

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Table 1. Concentrate and roughage feed composition offered from 16 to 23 monthly ages

Ingredients	Percentage of diet as fed	
Concentrate ingredients:		
Wheat	29.78	
Soybean meal	5.96	
Corn	39.70	
Wheat bran	23.82	
Mineral supplement	0.74	
Vitamin A, D and E[1]		
Trace minerals premix [2]		
Feed analysis (DM Basis):	Concentrate	Timothy hay
DM, %	88.21	90.8
TDN, %	73.44	53.8
CP, %	13.25	6.1
Ash, %	3.39	4.3

[1] Each vitamin contains 1,000 IU. [2] Trace minerals premix contained (ppm): Fe (66), Cu (7.46), Co (0.09), Zn (48), Mn (41), Se (0.2) and I (0.04).

ages (16, 17, 18, 19, 20, 22 and 23 months-old). Since the seven days data collection covered the period from late October to early November of 20th month, the 21st months was omitted. The commercial concentrate and roughage feed composition are shown in Table 1. The feeds were given twice daily at 900 and 1500 h. Across monthly ages body weight was measured at 0800 h in day 1 and 8 of feed data collection. Blood was sampled on day 8 through the jugular vein at 0800 and 1400, i.e. before the feed was offered. Morning and afternoon plasma were pooled for blood analysis. All experimental animals were treated according to the "Guidelines for Care and Use of Experimental Animals of the Obihiro University".

Measurement of back fat thickness

Ultrasound (Aloka SSD338, Japan) was used for BFT determination at the 6 to 7th and 12 to 13th rib every month. At similar location below the lumbar column of the left torso the probe was placed consistently throughout the measurement across the monthly ages. The hair on skin was clipped and 'Konnyaku' a firm gelatinous pad was used to avoid deformation of skin surface when pressed, allowing visual accuracy of BFT. Mineral oil was liberally applied between the skin surface and 'Konnyaku and the probe to ensure proper contact and propagation of the sound waves. Real-time ultrasound images (5.0 MHz) on the monitor were recorded in videotape and photo-printed. The BFT was measured using micro-caliper with lowest calibration of 0.05 mm. The procedure on BFT measurement was patterned according to the guidelines of Brethour (1992).

Blood analysis

The blood samples from each animal were analyzed for plasma leptin using modified multi-species leptin RIA (Linco, St. Charles, MO) as described in the protocol, with

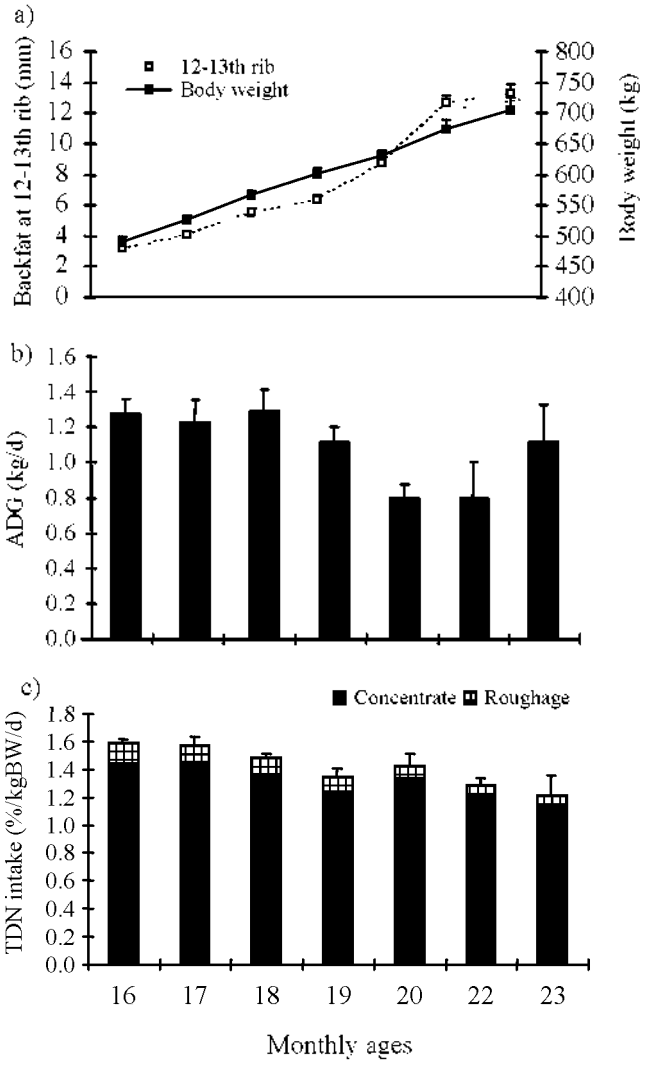

Figure 1. Means±SEM of (a) backfat thickness at 12 to 13th rib and body weight, (b) average daily gain and (c) TDN intake per kg BW of Holstein steers from concentrate (■) and roughage (□) feed across monthly fattening stage. Open (□) and solid (■) squares represent backfat thickness at 12 to 13th rib and body weight, respectively.

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 pooled 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

Linear regression analysis was used to determine relationship between dependent (TDN intake, plasma leptin,

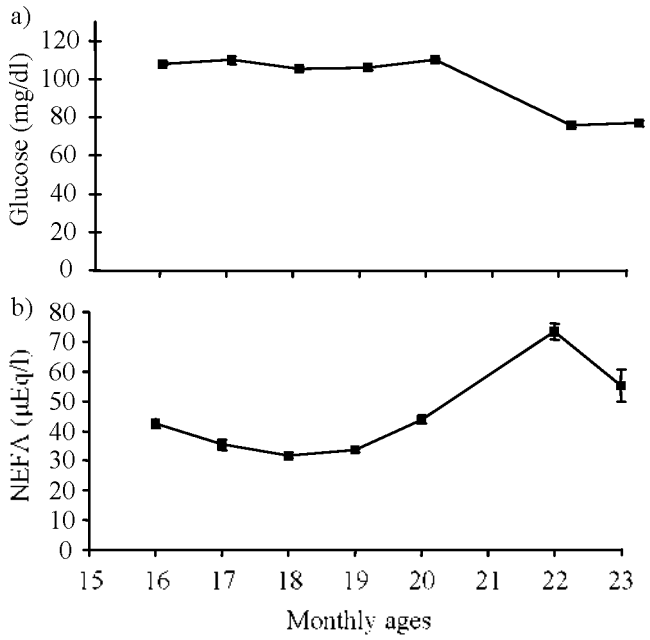

Figure 2. Means±SEM of plasma (a) glucose and (b) NEFA across the monthly fattening stage of Holstein steers.

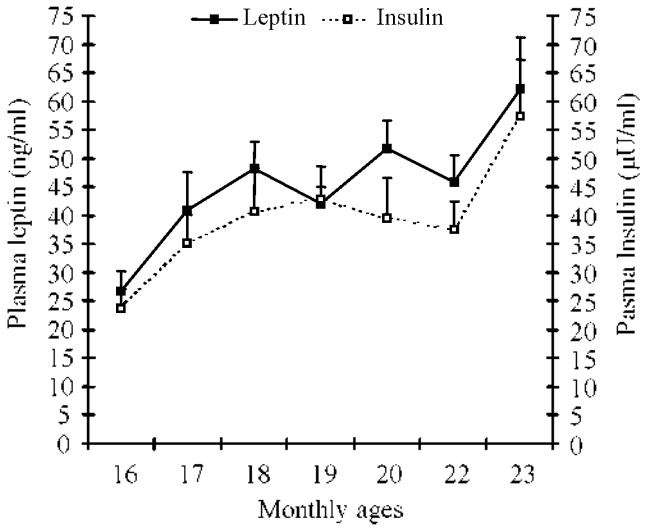

Figure 3. Means±SEM of plasma leptin and insulin are represented by solid (■) and open (□) squares, respectively across the monthly ages of Holstein steers.

and plasma insulin) and independent variables (body weight, BFT at 6 to 7th and 12 to 13th rib and ADG). Correlation analysis was used for the determination of association of TDN intake to plasma leptin and insulin, as well as between plasma leptin and insulin. The declared significant level was set at 5% ($p \leq 0.05$) and 10% ($p \leq 0.10$) level. GLM (General Linear Model) of the SAS system statistical software was utilized for simple linear regression and correlation analysis (SAS, 1998).

RESULTS

Figure 1 shows the changes in (a) BFT at 12 to 13th rib and body weight, (b) ADG and (c) TDN intake per kg BW across monthly ages. Backfat thickness revealed parallel increase with body weight across monthly ages. The ADG and TDN intake was constantly high from 16 to 18 months old, but the ADG slightly declined from 19 to 22 months old with compensatory gain at 23rd mo, while TDN intake decreased noticeably from 18 to 23 months old. *Ad libitum* and high grain fed Holstein steers showed gradual decline in TDN intake per kg BW from 16 to 23 months old (from 1.59% to 1.21% per kg BW) with about -24% decrease in TDN intake. The decline in TDN intake partly contributed to the decrease in ADG at the latter half of the finishing period.

Changes in some blood metabolites across the selected monthly ages are shown in Figure 2. Plasma glucose was constantly high from 16 to 20 months old and it was depressed at 22 and 23 months old. Plasma NEFA showed similar concentration from 16 to 20 months old, but higher levels were observed at 22 and 23 months old. Plasma total cholesterol and triglyceride did not show changes with monthly ages (data not shown).

Plasma leptin and insulin concentrations showed an increase from 16 to 23 monthly ages (Figure 3). Plasma leptin increased from 27 to 62 ng/ml, about 1.3 fold. The elevation of plasma leptin closely mimicked the elevation of plasma insulin, which resulted to a highly significant correlation between the two hormones (Table 2). Since plasma leptin and insulin are recognized to cause feed intake inhibition in sheep (Deetz, 1980; Deetz 1981; Morrison, 2001), we determined the correlation of TDN to plasma leptin and insulin. The age-related reduction of TDN intake was negatively associated to plasma leptin ($r=-0.49$, $p<0.004$) and plasma insulin ($r=-0.41$; $p<0.02$).

The correlation coefficients (r), slope and statistical values (p) of TDN, plasma leptin and insulin against body weight, backfat thickness (6 to 7th and 12 to 13th rib) and ADG and are shown in Table 2. The data reveals significant positive relationship of plasma leptin to BW and BFT at 6 to 7th and 12 to 13th rib. Plasma leptin was not a good indicator of ADG as it shows non-significant relationship. Plasma insulin shows positive but weaker relationship with BFT compared to plasma leptin.

DISCUSSION

The age-related changes in plasma leptin showed an increase in trend at fattening period, suggesting parallel increase with body fat deposition (Vega et al., 2002), hence this study was performed to substantiate the suggested parallel increase of plasma leptin and backfat and to include

Table 2. Correlation coefficients (r) with corresponding level of significance (p) of castrated Holstein steers between various measurements from 16 to 23 monthly ages

Variables	r	Slope	Significance (p)
Plasma leptin (ng/ml)			
vs. Body weight (kg)	0.56	0.092	0.0010
vs. BFT at 6 to 7th rib[1] (mm)	0.50	2.15	0.0034
vs. BFT at 12 to 13th rib[1] (mm)	0.45	1.73	0.0096
vs. ADG (kg/d)	-0.28	-11.10	0.1220
vs. Plasma insulin	0.54	0.44	0.0013
Plasma insulin (μU/ml)			
vs. Body weight (kg)	0.30	0.061	0.1022
vs. BFT at 6 to 7th rib[1] (mm)	0.36	1.62	0.0406
vs. BFT at 12 to 13th rib[1] (mm)	0.32	1.27	0.0761
vs. ADG (kg/d)	-0.22	-11.62	0.2005
Total digestible nutrient (TDN)			
vs. Body weight (kg)	-0.62	-0.002	0.0002
vs. BFT at 6 to 7th rib[1] (mm)	-0.37	-0.025	0.0321
vs. BFT at 12 to 13th rib[1] (mm)	-0.48	-0.029	0.0052
vs. ADG	0.30	0.185	0.0971
vs. Plasma insulin	-0.41	-0.005	0.0173
vs. Plasma leptin	-0.49	-0.0077	0.0042

[1] BFT means backfat thickness (mm).

plasma leptin relationship with TDN intake of steers across monthly ages, offered constantly with high concentrate and roughage feed from 16 to 23 monthly ages.

High grain-fed condition at fattening period was performed to facilitate maximum body fat accumulation. In the experiment of Loerch (1991), 85% concentrate plus 15% corn silage diet obtained significantly higher ADG, DM intake and feed/gain ratio compared to groups fed with 100% concentrate diet. Kreikemeier et al. (1990) also supported that the optimum level of roughage necessary to obtain best animal performance was within 5 to 10% in high concentrate diet. In our study, the volume of roughage consumed declined from 13% to 5% of the diet (Figure 1), and this may have caused the sufficient physical stimulation of ruminal epithelial surface for maximum animal performance as manifested by high ADG (Figure 1).

The decreasing ADG at latter half of monthly ages cannot be attributed solely to the reduction in TDN intake, because the proportion of TDN intake and ADG was perceived higher from 19 to 22 months old, indicating increased energy requirement per kg body weight gain. Insulin's central effect on feed intake remain controversial (Ikeda et al., 1986), hence its involvement in TDN intake reduction with monthly ages was considered part of adipose metabolism. However, plasma leptin involvement in TDN intake cannot be concluded in this study, because this experiment only shows the pattern of relationship of various variables associated with plasma leptin at fattening period. Research at micro-level such as leptin treatment needs to be conducted to specifically clarify their role in the age-related TDN intake reduction of finishing steers offered with high grain diet.

Blood metabolites manifest its availability or utilization and may indicate animal's nutritional condition. High preprandial plasma glucose from 16 to 20 months old confirmed high grain-fed consumption and the drop to normal levels during the last two monthly ages implied glucose utilization necessary for acclimatization for winter season, this can be supported by poor performance of cattle in cold season reported by Birkelo et al. (1991). Likewise the elevated preprandial plasma NEFA obtained consistently reflects the actual body lipid loss because of acclimatization. In this study, the level of plasma NEFA was generally lower than those reported in similar breed by Matsuzaki et al. (1997). The lower NEFA concentration in our experiment can be explained by higher level of grain consumption (2.3 vs. 1.0%/kg BW).

The BFT at 12-13th rib of the experimental steers observed from 490 to 704 kg bodyweight varied from 3 to 15 mm with fattening. Wells and Preston (1998) shows similar results in two different breeds (i.e. *Bos Taurus* and *Bos indicus*) of steers at similar fattening period. Brethour (1992) reported the accuracy and repeatability of ultrasound in measuring BFT in numerous cattle and Houghton and Turlington (1992) affirmed its accuracy in other livestock. Compared to actual measure of BFT the ultrasonic measures were 8% or about 1.2 mm lower (Brethour, 1998). Ultrasound can be used to track live animal changes in absolute BFT (Brethour, 1992; Houghton and Turlington, 1992). A little modification was included in our methodology, i.e. the utilizing 'Konnyaku' a firm gelatinous pad that can minimize error by absorbing pressure put upon the skin surface during ultrasound measurement. In film prints, the area covered by 'Konnyaku' display a faded dark area distinctly recognizable before the skin line. Ten different commercially available materials were tested in

our preliminary study and 20 mm thickness showed excellent visual results. Backfat may not have similar thickness in other portions, but our measurements at 6 to 7th and 12 to 13th ribs were comparable, indicating that either of the two BFT measures may represent general backfat thickness.

In humans, subcutaneous adipose tissue showed greater secretion of plasma leptin compared to omental adipose tissue (Hube et al., 1996; Russel et al., 1998), and plasma leptin showed significant relationship with subcutaneous adiposity at umbilicus level but not with visceral fat area (Takahashi et al., 1996). In sheep, backfat thickness to liveweight ratio showed significant linear relationship with plasma leptin (Blache et al., 2000a). The significant linear relationship between BFT and plasma leptin we observed in steer upholds the previous notion that the linear increase in plasma leptin at fattening period is associated to body fatness. The BFT at 6 to 7th and 12 to 13th rib generally showed observable bimonthly accumulation. However plasma leptin concentration rose more steeply compared to BFT from 16 to 18 months old, then it leveled-off from 18 to 22 months old and attained the highest concentration at the last month. Backfat thickness did not perfectly reflected the changes in plasma leptin, which may be explained by one or a combination of the following hypothesis; (1) backfat accumulation differs significantly with other portions of fat depots, (2) different portions of fat depots differ in degree of plasma leptin secretion and (3) leptin secretion and removal vary with monthly ages. Although significant association between plasma leptin and two BFT measurements was observed, BFT accumulation does not perfectly account for plasma leptin concentration in highly grain-fed Holstein steers.

In vitro experiment demonstrated that insulin increase leptin secretion from adipocyte or tissues of rat (Hardie et al., 1996; Barr et al., 1997; Siegrist-Kaiser et al., 1997), mouse (Rentsch and Chiesi, 1996; Leroy et al., 1996) and human (Russel et al., 1998). *In vivo* study in human showed a chronic effect of insulin on leptin production (Kolacynski et al., 1996), and hyperinsulinemia increased plasma leptin concentration after 4 h (Ultranian et al., 1996). However, insulin-induced increase in leptin was lower in obese insulin-resistant men (Saad et al., 1998), this effect of obesity was demonstrated in rats (Olefsky et al., 1975) and bovine (Vernon et al., 1985) indicating that decreased insulin binding was related to obesity and caused by a decreased number of insulin receptor site per cell. Briefly the results show that the stimulated-effect of insulin on leptin production decreases with insulin resistance and increasing adiposity, as highlighted by Saad (1998). Considering all these information the significant linear relationship of plasma insulin to BFT and to plasma leptin at fattening period support the following, (1) the concept of relative insulin resistance with increasing adiposity (Olefsky et al., 1975; Vernon et al., 1985; Saad et al., 1998) and age (Rowe et al., 1983); and (2) the involvement of endogenous insulin in plasma leptin secretion from adipocyte. Plasma leptin's follow through on plasma insulin level across the monthly ages may represent adipose metabolism. Although the age-related increase in backfat thickness did not perfectly mimic plasma leptin and insulin level, BFT obtained significant relationship with both hormones. Furthermore, the persistence of plasma leptin mimic on plasma insulin level may be explained by the blunting of adipose tissue insulin resistance with increasing adiposity and age through progressive increase in endogenous insulin.

The noticeable elevation of plasma leptin from 19 to 22 monthly ages compared to 16 month old coincided with lower TDN intake/kg BW at latter period. Although BFT accumulation did not slow down within this period, there is possibility that visceral organs and its adipose tissue was attenuated during reduced ADG at latter period. Some authors reported that internal organs of the body are sensitive to changes in nutrition, possibly because they are metabolically active than muscle and fats (Koong et al., 1985; Hornick, 2000). The long-term reduction in TDN intake per kg BW across monthly ages in this study suggests an involvement of plasma leptin. In sheep, increase in plasma leptin due to nutrition also increased cerebrospinal leptin concentration (Blache et al., 2000a), and the central infusion of leptin in ewe (Morrison et al., 2001) and male sheep (Blache et al., 2000b) resulted to decrease in voluntary feed intake, however in an undernourished ewe lamb there was no observed reduction in feed intake (Morrison et al., 2001). In this study the TDN intake decreased from 1.6 to 1.2% per kg BW, and most likely if the offered TDN was maintained at moderate level, i.e. 1.2% per kg BW across the monthly ages, plasma leptin maybe attenuated and no related decline in TDN intake may follow. It seems that increased and sustained elevated level in plasma leptin is necessary to effect feed intake reduction, which needs further confirmations. Negative associations of TDN intake with plasma leptin and BFT during finishing period suggest long-term involvement of adipose tissues in the feed intake regulation of steers fed high concentrate diet. The possible effect of associated plasma leptin and insulin elevation on feed intake regulation may be within 24% at finishing period.

IMPLICATIONS

Recognizing the long-term involvement and central action of leptin on feed intake and the results of our experiment, it is suggested that (1) increased and sustained elevated level in plasma leptin is necessary to effect feed

intake reduction and (2) 1.2% TDN intake per kg BW level of feeding or lower will not cause significant reduction on feed intake of finishing steers. Limiting the feed to 1.2% TDN per kg BW would be sufficient to eliminate the associated possible effect of plasma leptin and insulin on feed intake, favorable to minimize leftover feeds and reduce cost.

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