

The effect of feeding frequency on the hormonal profile, carcass characteristics, and feedlot performance in Iranian Holstein calves

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Abstract: The aim of the present study was to investigate the effect of feeding frequency on body weight, carcass characteristics, and hormonal profile in Iranian Holstein calves. The study included 12 Holstein bull calves that were assigned to 2 groups according to initial live body weight. The animals were fed a TMR ration based on live body weight. The calves were fed a standard diet for 31 weeks; control calves were fed twice a day and treatment calves were fed 7 times a day. Live body weights were recorded every 21 days. Blood samples were collected 1 h after the final feeding and every 4 h during the last 24 h of the experiment, and were analyzed for plasma leptin and insulin concentrations. Four calves from each group were slaughtered and carcass characteristics were measured. Plasma leptin concentrations were higher ($P < 0.01$) between midnight and early morning in the treated group than in the control group. Mean plasma insulin concentration was lower ($P < 0.01$) in the treated calves than in the control calves. Mean internal fat content and depth of subcutaneous fat were ($P < 0.05$) higher in the control group than in the treated group, while other carcass characteristics did not significantly differ between the 2 groups. It was concluded that increasing the feeding frequency of fattening Holstein calves decreased fat reservoirs, which was probably accompanied by plasma leptin and insulin changes.

Key words: Feeding frequency, leptin, insulin, carcass characteristic, calf

Introduction

Increasing the feeding frequency may improve bioenergetic efficiency and nitrogen retention in the body (1,2). Feeding animals less frequently has been shown to increase fat deposition and mobilization; consequently, decrease the efficiency of energy retention (3). Previous studies have shown that feeding cows 6 or 7 times a day instead of 2 times a

day increased dry matter intake, crude protein intake, and rumen pH, whereas it decreased the rumen propionate:acetate ratio (4-6). Moreover, plasma insulin levels tended to decrease (4).

Increasing the feeding frequency in cattle decreased mean plasma insulin and glucagon concentrations, and consequently prevented a reduction in milk fat when the cattle were fed high

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concentrate rations (3). These researchers also indicated that feeding cows 6 times a day instead of 2 times a day decreased insulin, fatty acids, and butyrate levels, and increased GH and glucose concentrations (7). These changes may prevent reductions in milk fat. Consuming easily digestible carbohydrates will decrease rumen pH and, consequently, cellulolytic activity (8). In contrast, it can increase amylolytic activity and the rumen propionate:acetate ratio (8,9). Increases in the propionate concentration stimulate insulin secretion in ruminants (10), which then stimulates glucose and fatty acid absorption, consequently increasing lipogenesis and reducing lipolysis in adipose tissue. In lactating cows insulin usually decreases the availability of fatty acids for milk fat synthesis (11). Therefore, increasing the feeding frequency probably reduces fat synthesis and deposition in the body.

Leptin, a hormone that is primarily secreted by adipose tissue, has a direct effect on body fat deposits (12). Leptin increases lipolysis (13) and thermogenesis (14), whereas the plasma leptin concentration can be affected by feeding (15,16). Hence, leptin apparently mediates the influence of feeding frequency on lipogenesis and fat deposition; however, there is no evidence to support this relationship. The aim of the present study was to investigate the effect of feeding frequency on leptin and insulin levels, body weight, and carcass characteristics in growing Holstein bull calves.

Materials and methods

Animals and location

This study was conducted in 2005 and 2006 at the research station of the Department of Animal Science, University of Tehran, Karaj. The study included 12

Iranian Holstein bull calves of similar age (4 months ± 2 weeks).

Experimental design

The calves were assigned to 2 groups (n = 6) according to initial body weight. Animals in the control group were fed 2 times a day (at 0800 and 2000), whereas animals in the treatment group were fed 7 times a day (at 0000, 0400, 0800, 1200, 1600, 2000, and 2400); both groups were fed a standard diet for 31 weeks. The diets were prepared to meet the animals' requirements based on NRC (17). Calves were fed according to a percentage of their body weight. The composition of the diet is shown in Table 1. Body weight was measured every 21 days after 16 h fasting. Blood samples were collected 1 h after the final feeding (0100) and every 4 h during the last 24 h of the experiment (0500, 0900 1300, 1700, 2100, and 0100 the next day). The samples were centrifuged at 3000 rpm for 20 min, and then plasma was separated and frozen at -20 °C. Animals were slaughtered in a modern abattoir in which the conditions were strictly supervised and every effort was made to ensure that the procedure was humane.

Determination of carcass characteristics

Sixteen hours before slaughter rations were withheld and the calves were weighed. Then the carcass characteristics were recorded (18).

Hormone assay

Plasma leptin and insulin concentrations were measured by radioimmunoassay, using validated kits (Tabeshyare Nour Co., Tehran, Iran) in a single assay. The sensitivity of the leptin and insulin measurements was 0.1 ng mL⁻¹ and 0.3 mIU mL⁻¹, respectively. Intra-assay CV for leptin and insulin was 8% and 7%, respectively.

Table 1. Dietary ingredients of concentrate ration introduced to both control and treatment groups.

Ingredients	percentage	Ingredients	percentage
Barley	35	NaHCO ₃	1
Corn	15	Mineral-Vitamin premix	0.6
Wheat bran	12.3	Di Calcium Phosphate	0.2
Beet pulp	9	CaCO ₃	0.3
Fat	1.5	Common Salt	0.1
Cottonseed meal	12	Zeolite	3
Soybean meal	10		

Statistical analyses

Body weight, and leptin and insulin concentrations were analyzed using Proc MIXED, and weights of the carcass cuts were analyzed using the GLM procedure in SAS software (19). The MIXED model used in this study was as follows:

$$Y_{ij} = m + A_i + B_j + (AB)_{ij} + e_{ij}$$

where y_{ij} is body weight, or the observed leptin or insulin concentration, m is the mean, A_i is the treatment (feeding frequency) effect, B_j is the time effect (hours for hormones; days for weight), AB_{ij} is the treatment \times time effect, and e_{ij} is residual error. The GLM model used in current study was as follows:

$$Y_i = m + A_i + b(W_i) + e_i$$

where y_i is the observed weight of the carcass cuts, m is the mean, A_i is the treatment (feeding frequency) effect, b is the regression coefficient, W_i is initial body weight, and e_i is the random residual error. Means were compared by LS means and $P < 0.05$ was considered significant.

Results

Growth and carcass characteristics

Changes in body weight recorded every 21 days during the experimental period are presented in Figure 1. As shown, increasing the feeding frequency did not have a significant effect on body weight. The results indicate that internal fat content in the treatment group was significantly ($P < 0.05$) lower than in the control group, whereas the other carcass

parameters did not significantly differ between the 2 groups (Table 2).

Carcass quality in the control group and treatment group is presented in Table 3. Subcutaneous fat depth was significantly ($P < 0.05$) higher in the control than in the treatment group. A tendency for increased rib eye muscle area was observed in the treatment calves. Mean weights of carcass components are shown in Table 4, which indicate there were not any significant differences between the 2 groups.

Plasma leptin and insulin concentrations

The results show that the mean plasma leptin concentration was significantly ($P < 0.01$) higher in the treatment group than that in the control group ($6.63 \pm 0.99 \text{ ng mL}^{-1}$ and $3.36 \pm 0.63 \text{ ng mL}^{-1}$, respectively). The effects of time and treatment \times time

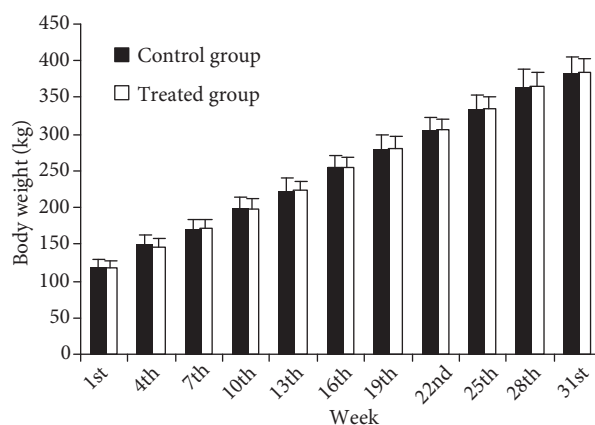


Figure 1. Mean (\pm SEM) body weight during the experimental period at 3-week interval.

Table 2. Mean (\pm SEM) weights of carcass offal in the control and treated groups.

Parameter	Group		Parameter	Group	
	Control	Treated		Control	Treated
Internal fat	4.56 \pm 0.80 ^a	3.38 \pm 0.20 ^b	Liver	1.63 \pm 0.02	1.78 \pm 0.07
Head	4.67 \pm 0.17	4.6 \pm 0.13	Spleen	0.22 \pm 0.01	0.19 \pm 0.02
Limb	2.16 \pm 0.11	2.13 \pm 0.08	Lungs	1.37 \pm 0.11	1.78 \pm 0.02
Skin	10.05 \pm 0.39	9.72 \pm 0.43	Heart	0.49 \pm 0.03	0.55 \pm 0.03
Full rumen	11.9 \pm 0.81	12.6 \pm 0.66	Kidney	0.3 \pm 0.01	0.3 \pm 0.02
Empty rumen	4.59 \pm 0.17	4.55 \pm 0.27	Testis	0.16 \pm 0.01	0.18 \pm 0.03

Values without a common letter in each row are significantly different ($P < 0.05$).

Table 3. Mean (\pm SEM) weights of carcass characteristics in the control and treated groups.

Parameter	Group	
	Control	Treated
Live body weight (kg)	406.5 \pm 27.91	390.5 \pm 18.46
Empty body weight (kg)	365.5 \pm 8.22	350.50 \pm 20.30
Hot carcass weight (kg)	223.6 \pm 6.76	218.7 \pm 11.84
Dressing percentage	55.01 \pm 1.40	56.00 \pm 0.40
Hot carcass weight (%)	61.18 \pm 1.5	60.40 \pm 0.30
Carcass length (cm)	143.5 \pm 7.30	139.25 \pm 6.34
Rib eye muscle area (cm ²)	129.25 \pm 4.23	133.25 \pm 6.7
Depth of subcutaneous fat of loin muscle (mm)	4.23 \pm 0.192 ^a	1.90 \pm 0.619 ^b

Values without a common letter in each row are significantly different ($P < 0.05$).

Table 4. Mean (\pm SEM) of carcass cuts of calves in the control and treated groups.

Parameter	Group	
	Control	Treated
Carcass cuts weight (kg)		
Neck	14.5 \pm 0.7	15.42 \pm 0.5
Shoulder	42.77 \pm 2.8	48.35 \pm 3.2
Loin	34.62 \pm 2.6	35.72 \pm 3.1
Round	74.75 \pm 3.9	75.87 \pm 2.1
Breast	44.47 \pm 1.7	48.97 \pm 3.4
In proportion to live body weight		
Neck	3.6 \pm 0.0	3.79 \pm 0.2
Shoulder	11.89 \pm 0.6	12.24 \pm 0.2
Loin	8.78 \pm 0.6	8.87 \pm 0.2
Round	18.66 \pm 1.4	19.14 \pm 0.2
Breast	11.87 \pm 0.8	12.16 \pm 0.3
In proportion to hot carcass weight		
Neck	6.47 \pm 0.4	6.89 \pm 0.0
Shoulder	21.81 \pm 0.4	21.62 \pm 1.2
Loin	15.83 \pm 0.3	15.97 \pm 0.9
Round	34.18 \pm 0.2	33.93 \pm 0.4
Breast	20.34 \pm 0.6	21.57 \pm 1.4

were also significant ($P < 0.01$). It was also observed that plasma leptin concentrations were significantly higher early in the morning in the treatment group than those in the control group. Fluctuations in plasma leptin concentration every 4 h during the last 24 h of the study are shown in Figure 2.

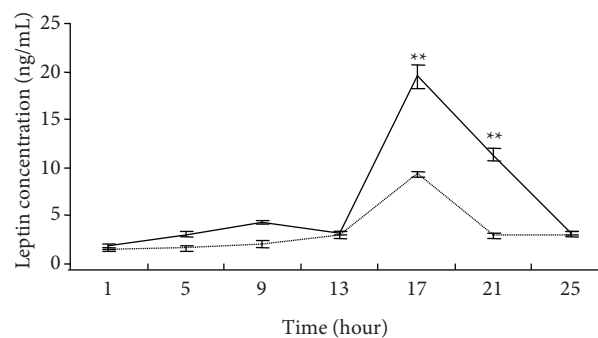


Figure 2. Mean (\pm SEM) of plasma leptin concentrations during 24 h at 4 h interval in the control (dashed line) and treated (solid line) groups. ** ($P < 0.01$)

The results also show that the mean plasma insulin concentration was significantly ($P < 0.01$) higher in the control group than in the treatment group (15.03 \pm 1.33 mLU mL⁻¹ and 12.84 \pm 0.95 mLU mL⁻¹, respectively). Fluctuations in the plasma insulin concentration every 4 h during the last 24 h of the study are presented in Figure 3.

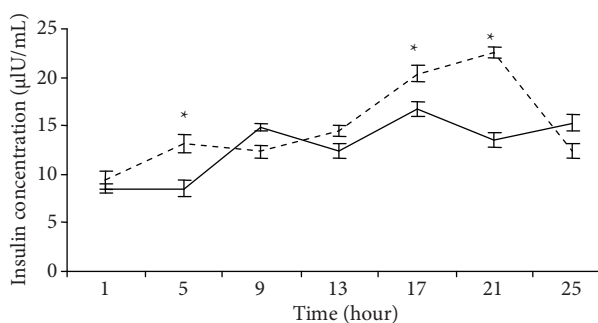


Figure 3. Mean (\pm SEM) of plasma insulin concentrations during 24 h at 4 h interval in the control (dashed line) and treated (solid line) groups. * ($P < 0.05$)

Discussion

In the present study increasing the feeding frequency significantly decreased internal fat and

subcutaneous fat depth in Holstein calf carcasses. The ratios of some carcass components were higher in the treatment group than in the control group, but the differences were not significant. Additionally, mean body weight was not significantly different between the 2 groups. It has been suggested that increasing the feeding frequency decreases fat synthesis and deposition in the body (1). Sutton et al. (3,7) in 2 separate studies, observed that feeding dairy cows 6 times a day instead of 2 times a day decreased the plasma insulin concentration, and increased growth hormone and glucose concentrations. Consequently, increasing the feeding frequency could prevent reductions in milk fat in cattle that consume a high concentrate ration (3). French and Kennely (6) also reported that increasing the feeding frequency improved rumen pH, but decreased the propionate:acetate ratio, and plasma insulin and glucagon concentrations. These researchers suggest that the effects observed were due to a decrease in the rumen propionate concentration. Considering the important role of insulin in lipogenesis and reducing lipolysis, and the stimulatory effect of growth hormone on lipogenesis and protein accretion (11), the researchers concluded that increasing the feeding frequency could decrease fat synthesis and deposition via changes in insulin or growth hormone concentrations. In the present study increasing the feeding frequency decreased insulin levels and fat deposition in fattening calves. Therefore, our results confirm the role of insulin in fat deposition in calves.

Increasing the feeding frequency in the present study increased the mean plasma leptin concentration at 0000 in the treatment group, as compared to the control group. Interestingly, this effect was only observed in the nocturnal leptin peak. Studies of some species have shown that leptin secretion has a circadian rhythm and that its peak occurs between 0000 and early morning (20). The present study's results are in agreement with these studies.

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Leptin is secreted by white adipose tissue and is an adipostatic factor (21). The concentration of this hormone in most mammals, especially ruminants, has a direct relationship with feed consumption (15). Frühbeck et al. (13,22) reported that leptin can increase lipolysis in vitro and in vivo in ob/ob mice. Moreover, leptin has been shown to stimulate thermogenesis (23,24). Rouru et al. (14) reported that intravenously infused leptin increases insulin-stimulated glucose utilization and favors the expression of uncoupling proteins in brown fat tissue; therefore, leptin has lipolytic and thermogenic effects in the body.

The depth of subcutaneous fat and internal fat content decreased in the treatment group. Hence, the increase in the mean plasma leptin level in the treatment group that was observed at the nocturnal peak and was maintained for several hours may have contributed to the observed decrease in carcass fat deposition. Moreover, the rib eye muscle area tended to increase in the treatment group, which resulted in a trend toward increased muscle tissue accretion in the carcasses. Therefore, we think that leptin acted as a partitioning hormone, which mobilized energy and nutrients from adipose tissue toward muscle tissue. These results suggest that increasing the feeding frequency could improve bioenergetic efficiency and nitrogen retention in fattening calves, such as dairy cows (1).

In conclusion, increasing the feeding frequency in feedlot Holstein bull calves led to increased leptin and decreased insulin-circulating concentrations in the blood. Moreover, increasing the feeding frequency significantly decreased subcutaneous and internal fat. Hence, it seems that the effects of increasing the feeding frequency on carcass fat content may have been mediated by increased leptin and reduced insulin concentrations. The role of other hormones, such as growth hormone and glucagon, should not be ignored and warrants further investigation.

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