Effect of dietary vitamin A restriction on marbling and conjugated linoleic acid content in Holstein steers

M. A. Gorocica-Buenfil, F. L. Fluharty, C. K. Reynolds, and S. C. Loerch[1]

Department of Animal Sciences, The Ohio State University, Wooster 44691

ABSTRACT: To determine the effect of duration of dietary vitamin A restriction on site of fat deposition in growing cattle, 60 Holstein steers (BW = 218.4 ± 6.55 kg) were fed a diet based on high-moisture corn, with 2,200 IU of supplemental vitamin A/kg of DM (control) or no supplemental vitamin A for a long (243 d; LR) or short (131 d; SR) restriction before slaughter at 243 d. The SR steers were fed the control diet for the first 112 d. Steers were penned individually and fed for ad libitum intake. Jugular vein blood samples for serum retinol analysis were collected on d 1, 112, and 243. Carcass samples were collected for composition analysis. Subcutaneous fat samples were collected for fatty acid composition. Fat samples from the i.m. and s.c. depots were collected to measure adipocyte size and density. Feedlot performance (ADG, DMI, and G:F) was not affected ($P > 0.05$) by vitamin A restriction. On d 243, the i.m. fat content of the LM was 33% greater ($P < 0.05$) for LR than for SR and control steers (5.6 vs. 3.9 and 4.2% ether extract, respectively). Depth of backfat and KPH percentage were not affected ($P = 0.44$ and 0.80, respectively) by vitamin A restriction. Carcass weight, composition of edible carcass, and yield grade were similar among treatments ($P > 0.10$). Liver retinol (LR = 6.1, SR = 6.5, and control = 44.7 μg/g; $P < 0.01$) was reduced in LR and SR vs. control steers. On d 243, LR and SR steers had similar serum retinol concentrations, and these were lower ($P < 0.01$) than those of control steers (LR = 21.2, SR = 25.2, and control = 36.9 μg/dL). Intramuscular adipose cellularity (adipocytes/mm^2 and mean adipocyte diameter) on d 112 and 243 was not affected ($P > 0.10$) by vitamin A restriction. Restricting vitamin A intake for 243 d increased i.m. fat percentage without affecting s.c. or visceral fat deposition, feedlot performance, or carcass weight. Restricting vitamin A intake for 131 d at the end of the finishing period appears to be insufficient to affect the site of fat deposition in Holstein steers.

Key words: Holstein, vitamin A, marbling

J. Anim. Sci. 2007. 85:2243–2255
doi:10.2527/jas.2006-781

INTRODUCTION

Beef carcass value is influenced by the site of fat deposition. Fat deposited i.m. (marbling) increases carcass value, whereas fat deposited in the s.c. depot diminishes it (USDA, 1997). The mechanisms controlling the site of fat deposition are not well understood.

Adipocyte differentiation is important for i.m. fat deposition because during the finishing period this depot grows largely by hyperplasia, whereas the s.c. depot grows predominantly by hypertrophy (Cianzio et al., 1985). We recently reported that feeding low-vitamin A diets to beef steers appears to increase adipocyte differentiation in the i.m. depot without affecting s.c. adipocytes. This was accompanied by numerical increases in marbling scores and USDA carcass quality grades, with no effects on backfat deposition and USDA yield grades (**YG**; Gorocica-Buenfil et al., 2007). Marbling scores also were increased when low-vitamin A diets were fed to Japanese Black cattle (Adachi et al., 1999). The duration of vitamin A restriction required to improve i.m. fat deposition remains unknown. It is likely that to affect the vitamin A status of the animal, hepatic vitamin A stores need to be depleted. However, research in this area is negligible.

The effect of feeding low-vitamin A diets on beef fatty acid composition remains unclear. The enzymatic activity of stearoyl coA desaturase (**SCD**), required for the endogenous synthesis of CLA in ruminants, may be reduced by retinol (Alam and Alam, 1985). Feeding low-vitamin A diets may increase SCD activity, thus increasing the CLA content in beef. Only limited data are available reporting the effect of dietary vitamin A restriction on the fatty acid composition and CLA content of beef.

[1]Corresponding author: loerch.1@osu.edu
Received November 27, 2006.
Accepted April 25, 2007.

The objectives of this experiment were to 1) determine the effect of duration of vitamin A restriction on the site of fat deposition in Holstein steers; and 2) investigate the effects of dietary vitamin A restriction on fatty acid composition and CLA content of beef.

MATERIALS AND METHODS

All procedures involving animals were approved by The Ohio State University Agricultural Animal Care and Use Committee.

A feedlot trial was conducted at The Ohio State University Beef Center in Wooster to evaluate the effect of duration of vitamin A restriction on the site of fat deposition and the fatty acid profile of beef. Holstein steers were purchased in Ohio by a cattle order buyer. Holstein steers (n = 70, BW = 175 ± 4.5 kg) were used to extend the dietary vitamin A restriction by taking advantage of their slower growth rate and lighter initial BW compared with beef breeds.

Upon arrival at the feedlot, steers were vaccinated for infectious bovine rhinotracheitis, parainfluenza-3, *Haemophilus somnus*, *Pasteurella*, and *Clostridia* (Quadraplex, Somnugen 2P, and Dybelon; Bioceutic, St. Joseph, MO), and dewormed with Ivomec pour-on (Merial, Duluth, GA). Steers were revaccinated 14 d later.

Steers were penned and fed individually in a totally enclosed feedlot barn (slatted concrete floor, metal gates, 2.6 × 1.5 m each) during the experiment. For the first 45 d in the feedlot, the steers were fed an adaptation diet (60% corn silage). This diet was calculated to provide 2,700 IU of vitamin A/kg of DM. After adaptation was completed, the steers were weighed on 2 consecutive days to determine their initial BW, and the second initial weighing day was considered d 1 of the experiment.

Steers were randomly distributed to one of the following treatments, where d indicated the length of dietary vitamin A restriction: control = 0 d, short restriction (**SR**) = 131 d, and long restriction (**LR**) = 243 d. The control group received a high-moisture, corn-based diet supplemented with 2,200 IU of vitamin A/kg of DM for the duration of the experiment; the SR group was fed the control vitamin A diet for the first 112 d of the experiment and then was switched to the low-vitamin A diet (no supplemental vitamin A added to the diet; basal diet content estimated at 950 IU of vitamin A equivalents/kg of DM) for the remainder of the trial; and the LR group received the low-vitamin A diet for the duration of the experiment. The vitamin A content of the basal diet was calculated using NRC (1996) values. Feed samples were not analyzed to confirm the calculated values. Liver and serum samples were utilized as a biological indicator of vitamin A intake (Pyatt and Berger, 2005).

Diets contained high-moisture corn, corn silage, ground wheat straw, and a protein, mineral, and vitamin supplement (Table 1). Except for the vitamin A concentration, the experimental diets were identical.

Table 1. Diet composition

Item	Diet: Vitamin A restricted	Diet: Control
	% of DM, unless indicated otherwise	
Ingredient		
Corn, high moisture	75.00	75.00
Corn silage	5.00	4.99
Wheat straw	5.00	5.00
Soybean meal 44%	11.72	11.72
Urea	0.90	0.90
Limestone	1.35	1.35
Trace mineral salt[1]	0.46	0.46
Vitamin A, 30,000 IU/g	—	0.009
Vitamin D, 3,000 IU/g	0.009	0.009
Vitamin E, 44,000 IU/g	0.027	0.027
Selenium, 201 mg/kg	0.046	0.046
Rumensin-80[2]	0.016	0.016
Tylan-10[2]	0.046	0.046
Dynamate[3]	0.33	0.33
Animal-vegetable fat	0.10	0.10
Nutrient composition		
CP, %	14.5	14.6
Vitamin A,[4] IU/kg	950	3,150
Calcium,[4] %	0.54	0.54
Phosphorus,[4] %	0.33	0.33
Potassium,[4] %	0.68	0.68
NE_m,[4] Mcal/kg	2.11	2.11
NE_g,[4] Mcal/kg	1.44	1.44

[1]Contained > 93% NaCl, 0.35% Zn, 0.28% Mn, 0.175% Fe, 0.035% Cu, 0.007% I, and 0.007% Co.
[2]Elanco, Greenfield, IN.
[3]Magnesium sulfate and potassium sulfate, contained 22% S, 18% K, and 11% Mg (International Minerals and Chemical, Terre Haute, IN).
[4]Calculated using NRC (1996) values.

Steers were weighed every 28 d, and at the end of the experiment they were weighed on 2 consecutive days to determine final BW. Steers were weighed before feeding at 0800 and were not withheld from feed or water. Steers were implanted on d 1 and reimplanted on d 112 and 196. Synovex-S (20 mg of estradiol benzoate and 200 mg of progesterone; Fort Dodge Animal Health, Overland Park, KS) was used for all implant procedures.

Steers were offered feed once daily, beginning at 0800. Steers were fed for ad libitum intake, intake was recorded daily, and feedstuff samples were collected weekly to adjust diet formulations for changes in ingredient DM content and to determine DMI. Diet samples were collected biweekly and composited at the end of the trial for nutrient analysis. Composite feed samples were freeze dried, ground to pass a 1-mm screen, and analyzed for DM, OM, and N content (AOAC, 1996).

Blood samples (10 mL) were collected from the jugular vein of all steers on d 0, 112, and 243 to determine serum retinol levels. Tubes containing blood samples were immediately wrapped in aluminum foil to avoid

light damage to the retinol and were kept on ice until reaching the laboratory for harvest of serum. Serum was harvested by centrifuging the blood samples at 2,200 × *g* at 4°C for 10 min. Samples were frozen at –20°C until vitamin A analysis was performed (Ching et al., 2002).

Hepatic and s.c. fat vitamin A stores were determined. Subcutaneous fat and liver samples were collected at slaughter, snap-frozen in liquid N_2, immediately placed on ice, and protected from light damage. Samples were stored at –80°C before retinol analysis.

Serum vitamin A levels were analyzed according to the procedures of Weiss et al. (1995), with modifications. Briefly, samples were extracted with hexane, and the extracted samples were dried under N_2 gas at 37°C, reconstituted with ethanol, and injected into a HPLC equipped with a reverse phase column (Supelcosil LC-18, 25 cm × 4.6 mm, Supelco Inc., Bellefonte, PA). The solvent used was initially 75% water and 25% methanol (vol/vol) and then was changed linearly to 100% methanol over 2 min. The flow rate was 1.8 mL/min. All procedures were performed in the dark to avoid retinol light damage. The assay CV was less than 5%, and the limit of detection was 0.5 μg/dL.

Before retinol extraction, liver samples were saponified by heating them at 70°C for 10 min with 5 mL of a 50% KOH solution (Indyk, 1988). The samples were then extracted twice with hexane (10 mL each). The extracts were combined, and 5 mL of H_2O were added to allow for phase separation. After sample centrifugation at 2,200 × *g* for 10 min, a 5-mL aliquot of the extract was dried at 37°C under N_2 gas. The samples were reconstituted with ethanol and analyzed by HPLC, as described for serum samples.

Subcutaneous fat samples were saponified by heating them at 70°C for 10 min with 5 mL of 50% KOH. The samples were then extracted twice with 10 mL of hexane. After extraction, retinol in s.c. fat samples was analyzed by HPLC, as described for the liver samples. All-*trans* retinol obtained from Sigma Chemical Co. (St. Louis, MO) was used as the standard.

The diet consumed by the steers before acquisition was unknown, but the vitamin A status of the steers was evaluated by collecting serum samples from all steers on d 0. Additionally, 3 steers were randomly selected for slaughter to collect liver and s.c. fat samples at the initiation of the trial.

Carcass samples were also collected for composition and cellularity analysis. An intermediate slaughter was conducted to determine the effects of the duration of dietary vitamin A restriction on adipose tissue cellularity and carcass composition. On d 112, 3 steers fed the high- (2 steers from the control and 1 from the SR group) and 3 fed the low-vitamin A diet (LR group) were slaughtered. The cellularity of intramuscular and s.c. fat (adipocyte size and number), carcass characteristics, and body vitamin A stores in fat and liver were determined in the slaughtered steers. Samples from all of the remaining steers at the end of the trial were also collected and analyzed. Subcutaneous fat and hepatic vitamin A stores were determined, as previously described. Adipose tissue cellularity was determined for i.m. and s.c. depots.

The remaining steers were slaughtered when they reached 243 d on feed. Hot carcass weight, backfat thickness, LM area, and KPH % were determined by trained Ohio State University personnel. Carcass YG was calculated (USDA, 1997). Quality grade and marbling score were determined by a USDA official. Carcass characteristics were measured after a 48-h chill.

Samples of LM from the 9th to 11th thoracic rib of the right side of the carcass were collected, deboned, ground 3 times (Hobart model #4822, Hobart Co., Troy, OH), and analyzed for moisture, N, and ether extractable (**EE**) lipid content (AOAC, 1996). Final empty body composition of the edible carcass was determined using the procedures of Hankins and Howe (1946) and the equations of Garrett and Hinman (1969). Samples of LM from the 11th to 12th thoracic rib were collected, trimmed of external fat, ground 3 times, and analyzed for moisture, N, and EE (AOAC, 1996). Additionally, fatty acids in s.c. adipose tissue were extracted and methylated by alkaline transesterification and analyzed as described by Kramer et al. (1997). Methyl esters of fatty acids were separated on a 0.25-mm × 100-m, fused silica column (Supelco Inc.), using a Hewlett-Packard 5890 gas chromatograph, with automated injection and data reduction (HP 3365 Chemstation software, Hewlett Packard Co., Santa Clarita, CA). Standards for the CLA isomers *c*9, *t*11; *t*10, *c*12; and *t*9, *t*11 were obtained from Matreya Inc. (Pleasant Gap, PA).

Subcutaneous fat samples were collected on the kill floor, snap-frozen in liquid N_2, and transported on ice to the laboratory. Intramuscular adipose tissue was collected from the 6th to 8th thoracic rib after a 48-h chill. These samples were stored at –20°C until adipose cellularity and fatty acid composition were analyzed.

To determine adipocyte size and number, frozen adipose tissue samples were fixed and sectioned at a thickness of 6 to 8 μm in a CM1900 Leica cryostat (Meyer Instruments Inc., Houston, TX). The sections were stained with hematoxylin + eosin solution (Merck, Darmstadt, Germany) and mounted on Superfrost Plus slides (Fisher, Pittsburgh PA). Cell number and mean cell diameter were determined by computer image analysis (Image-Pro Plus, v. 4.5, MediaCybernetics Inc., Silver Spring, MD). Adipocyte presence in the samples was confirmed by staining an additional slide with Sudan IV (Culling, 1974).

The experimental data were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC). Serum retinol levels were analyzed for a completely randomized design with repeated measures. The model included the effects for treatment, days on feed at sample collection, and the treatment × days on feed interaction. The error structure used was ante dependence because it resulted in the lowest Bayesian criteria. Treatment

Table 2. Effect of the duration of dietary vitamin A restriction on feedlot performance of Holstein steers

	Treatment				
Item	Long restriction	Short restriction	Control	SEM[1]	*P*-value
No. of steers	16	17	18		
BW, kg					
Initial	218.1	218.2	219.0	6.7	0.99
Intermediate (112 d)	418.3	417.2	409.4	8.5	0.70
Final (243 d)	588.8	592.0	584.5	11.0	0.88
ADG, kg					
Initial (d 0 to 112)	1.79	1.78	1.70	0.45	0.43
Intermediate (d 112 to 243)	1.33	1.36	1.37	0.47	0.80
Total (d 0 to 243)	1.54	1.55	1.52	0.42	0.85
DMI, kg					
Initial (d 0 to 112)	7.93	7.65	7.43	0.19	0.16
Intermediate (d 112 to 243)	9.74	9.44	9.34	0.25	0.49
Total (d 0 to 243)	8.91	8.61	8.46	0.20	0.27
G:F, g/kg					
Initial (d 0 to 112)	225.4	232.4	229.1	4.7	0.57
Intermediate (d 112 to 243)	136.2	144.4	146.0	3.1	0.06
Total (d 0 to 243)	173.1	180.5	180.0	2.7	0.09

[1]For n = 16.

effects were partitioned into linear, quadratic, and cubic contrasts.

Animal performance, carcass characteristics, muscle fatty acid composition, and adipose cellularity data were analyzed as a completely randomized design. For the cellularity analysis, the fat depot (i.m. or s.c.) and the treatment × fat depot interaction were included in the model. Treatment means were compared using the PDIFF statement of SAS when protected by a significant ($P < 0.05$) *F*-value. Steer was used as the experimental unit for all statistical analyses.

RESULTS AND DISCUSSION

Animal performance data are presented in Table 2. Average daily gain, DMI, and G:F were not affected by vitamin A level at d 112 or at slaughter on d 243 (all $P > 0.05$). Steers in the LR treatment had a tendency ($P = 0.06$) for a reduction in G:F as a result of numerically greater DMI. It is unlikely that vitamin A restriction increased DMI. In a previous experiment (Gorocica-Buenfil et al., 2007) restricting dietary vitamin A for 168 d in Angus-based steers resulted in a trend for a slight reduction in ADG but no effects on DMI or G:F. In the present trial, gain was not affected by feeding low-vitamin A diets for as long as 243 d. Taken together, we concluded that removing vitamin A supplementation from corn-based finishing diets does not affect feedlot performance. The NRC (1976) established the requirement for vitamin A in feedlot cattle rations at 2,200 IU of vitamin A/kg of DM. This recommendation is based on sparse information published over 35 yr ago (Perry et al., 1965, 1968; Eaton et al., 1972) when modern techniques to assess vitamin A status (such as HPLC and mass spectrometry) were not utilized. Our results suggest that further research to determine the vitamin A requirements for feedlot cattle more precisely is warranted. Particularly, research is required to directly evaluate the effects of vitamin A restriction during the finishing period on animal health and immune status.

Hot carcass weight, LM area, backfat, and KPH were not affected by SR or LR (Table 3). Marbling scores were not affected by vitamin A restriction ($P = 0.36$). However, a numerical trend ($P > 0.10$) was observed in marbling score and the percentage of carcasses grading USDA Choice^{o} or above (from 28% in control to 50% in LR steers). If this effect were real, it would be economically important because most formulas used in the market to determine carcass value include a premium for carcasses ≥ Ch^{o} (USDA Agricultural Marketing Service, 2006). The numerical increase in the percentage of highly marbled carcasses is in agreement with our previous experiment (Gorocica-Buenfil et al., 2007) where we reported a 7% increase in the marbling scores when low-vitamin A diets were fed to Angus-based steers. For reasons unknown, the SR steers had the lowest marbling scores and percentage of ≥ Ch^{o} carcasses, indicating that vitamin A restriction for 131 d or less in Holstein steers is not sufficient to improve marbling scores.

Carcass YG was not affected ($P > 0.10$) by vitamin A restriction. Market grid formulas include severe discounts for carcasses with USDA YG of 4 or above. Considering that vitamin A restriction increased numerically the carcass quality grade while leaving HCW and YG unaffected, it is likely that LR carcasses would have a greater value than control or SR. To illustrate this,

Table 3. Effect of the duration of dietary vitamin A restriction on carcass characteristics of Holstein steers

	Treatment				
Item	Long restriction	Short restriction	Control	SEM[1]	*P*-value
No. of steers	16	17	18		
HCW, kg	344	345	340	7.7	0.86
Dressing %	58.3	58.3	58.1	0.41	0.86
Backfat, cm	0.81	0.78	0.68	0.073	0.44
LM area, cm^2	74.3	72.6	74.3	2.4	0.84
KPH, %	1.59	1.65	1.56	0.10	0.80
Marbling score[2]	634	571	602	31.5	0.36
Quality grade[3]	5.75	5.24	5.39	0.34	0.53
Select, %	19	25	22	10.7	0.53
$Choice^-$, %	31	29	50	12.3	0.39
$Choice^0$, %	25	18	11	10.0	0.59
$Choice^+$, %	6	12	6	6.7	0.77
Prime, %	19	6.0	11	7.8	0.53
≥$Choice^0$, %	50	35	28	12.2	0.42
Yield grade	2.81	2.88	2.62	0.14	0.36
Carcass value,[4] $/45 kg	136.98	134.62	137.03	1.9	0.67

[1]For n = 16.
[2]Marbling score: slight = 400 to 499, small = 500 to 599, modest = 600 to 699.
[3]4 = Select, 5 = $Choice^-$, 6 = $Choice^0$, 7 = $Choice^+$, 8 = Prime.
[4]Based on the grid price formula published by the USDA Market News Service for the week of 10/17/2005: Base price $137.24/45 kg ($Choice^-$, Yield grade 3.0); Premiums (per 45 kg): Prime = $7.70, $Choice^{0,+}$ = $1.93; Discounts (per 45 kg): Select = ($12.29); Yield grade 4.0 to 4.9 = ($13.90).

the value of each carcass was calculated using a grid price formula published by the USDA Market News Service for the week the animals were slaughtered (USDA Market News Service, 2005). On average, each LR carcass was valued $10.50 above control or SR carcasses. Although this difference was not statistically significant ($P > 0.10$), and it would vary depending on the current market prices, the economic relevance of this finding should not be ignored.

The effect of vitamin A restriction on edible carcass and LM composition is presented in Tables 4 and 5. Edible carcass EE increased ($P < 0.01$) as days on feed increased, whereas carcass OM and CP remained unchanged ($P > 0.05$) throughout the feeding period. Vitamin A restriction for 112 or 243 d did not affect carcass CP ($P = 0.53$ and 0.81 for d 112 and 243, respectively) or EE content ($P = 0.92$ and 0.17 for d 112 and 243, respectively). Vitamin A restriction for the first 112 d of the experiment did not affect intramuscular EE content ($P > 0.05$), although limited observations make this conclusion tenuous. Conversely, LM EE on d 243 was 33% greater ($P < 0.05$) in LR compared with SR

Table 4. Effect of dietary vitamin A restriction for 112 d on edible carcass and LM composition of Holstein steers

	Time on feed, d				
		112			
Item	0	Restricted[1]	Control[1]	SEM[2]	*P*-value[2]
No. of steers	3	3	3		
Edible carcass[3]					
DM	31.4 ± 1.64	41.1	41.7	1.81	0.82
OM	96.4 ± 0.27	97.7	97.0	0.28	0.15
Ether extract	11.0 ± 2.05	23.2	23.5	2.38	0.92
CP	16.8 ± 0.44	15.6	15.1	0.45	0.53
LM[3]					
DM	25.0 ± 0.96	25.9	25.7	0.66	0.86
OM	95.3 ± 0.68	96.3	96.6	0.56	0.72
Ether extract	1.0 ± 1.1	2.4	1.9	0.85	0.69
CP	19.3 ± 0.74	19.7	20.3	0.67	0.53

[1]Restricted = no supplemental vitamin A, and control = supplemented with 2,200 IU of vitamin A/kg of DM.
[2]SEM and *P*-value of R vs. control diet.
[3]As-is basis.

Table 5. Effect of dietary vitamin A restriction on edible carcass and LM composition at slaughter (d 243) of Holstein steers

	Treatment				
Item	Long restriction	Short restriction	Control	SEM[1]	*P*-value
No. of steers	16	17	18		
Edible carcass[2]					
DM	48.9	47.9	47.3	0.70	0.25
OM	97.9	97.7	97.7	0.11	0.15
Ether extract	33.2	31.8	30.9	0.86	0.17
CP	13.8	13.8	14.0	0.19	0.81
LM[2]					
DM	28.1	26.8	27.0	0.43	0.09
OM	96.6	96.4	96.3	0.30	0.71
Ether extract	5.6[x]	3.9[y]	4.2[y]	0.49	0.05
CP	19.1	19.3	19.1	0.32	0.80

[x,y]Within a row, means without a common superscript letter differ, $P < 0.05$.
[1]For n = 16.
[2]As-is basis.

and control steers. Thus, long vitamin A restriction specifically increased fat deposition in the i.m. depot, without promoting an increase in the overall fatness of the animal. We conclude that feeding low-vitamin A diets may be a feasible and economical strategy to affect the site of fat deposition within the beef carcass.

Pyatt and Berger (2005) hypothesized that the observed seasonal decline in carcass grade during the fall may be associated with previous high-vitamin A intake during spring and summer. Additionally, typical feedlot diets are formulated to provide 2 to 3 times NRC (1996) vitamin A recommendations (Galyean and Gleghorn, 2002). Thus, feedlot cattle in the United States are fed vitamin A in excess of their requirements. Results of this experiment provide evidence that the vitamin A level of the diet affects the site of fat deposition in feedlot cattle.

The lack of response to the SR treatment suggests that more than 131 d of vitamin A restriction may be necessary to lead to development of detectable increases in i.m. fat content. Alternatively, to affect the site of fat deposition in growing steers, it may be necessary for the vitamin A restriction to occur earlier in the finishing phase.

The similar CP and EE composition of the edible carcass among treatments is in agreement with the comparable YG calculated for these carcasses. Although differences in intramuscular EE were statistically significant, differences in marbling scores were not. Reasons for this apparent contradiction could be related to a greater variability in marbling scores, perhaps associated with the subjective nature of this measurement.

Increases in the LM fat content correspond to an enlargement of the i.m. fat depot. The i.m. depot development during the finishing phase appears to rely more on hyperplasia, the differentiation of new fat cells from preadipocytes (Cianzio et al., 1985). Conversely, the s.c. depot grows largely by hypertrophy, the enlargement of existing adipocytes (Hood and Allen, 1973). We have hypothesized that strategies that stimulate adipocyte differentiation during the feeding period might enhance the i.m. fat depot development relative to s.c. fat. Vitamin A inhibits adipocyte differentiation (Sato et al., 1980; Kumar et al., 1999). Thus, restricting vitamin A intake during the finishing period might release the inhibition on i.m. fat adipocytes to proliferate. We previously reported that greater marbling scores in steers fed low-vitamin A diets were accompanied by an increase in the number of adipose cells in the i.m. depot. This was interpreted as an indication that adipose hyperplasia was indeed stimulated (Gorocica-Buenfil et al., 2007). The effects of vitamin A restriction for 112 and 243 d on adipose cellularity are presented in Tables 6 and 7, respectively. The number of adipocytes per mm^2 and the mean cell diameter were not different ($P > 0.05$) among the LR, SR, and control steers on d 112 or 243 in the i.m. or the s.c. depots. This differs from results we reported previously when low-vitamin A diets were fed for 168 d. Based on muscle EE, cellularity changes suggesting an enlarged i.m. fat depot (more and smaller adipocytes suggesting hyperplasia) would be expected. However, adipocyte size and number were not different between R and control (d 112) or LR, SR, and control (d 243). Thus, the increased i.m fat content cannot be attributed to increased hyperplasia in response to vitamin A restriction. Nonetheless, on d 112, steers that were vitamin A restricted had numerically more and smaller i.m. adipocytes than control steers, which may suggest that a greater adipocyte differentiation was taking place in the R group. Furthermore, on d 112 R steers had 20% more ($P = 0.08$) adipocytes with a mean diameter of 75 to 85 μm adipocytes in the i.m. depot (Figure 1), which has been suggested as the breakpoint at which a new wave of i.m. adipocytes proliferate (Robelin, 1986; Schoonmaker et al., 2004). It can be speculated that by the time the steers were

Table 6. Effect of dietary vitamin A restriction for 112 d on the cellularity of adipose tissue of Holstein steers

	Time on feed, d						
		112			*P*-value[4]		
Item[1]	0	Restricted[2]	Control[2]	SEM[3]	D	F	D × F
Intramuscular depot							
No. of steers	3	3	3				
Cell number/mm^2	736 ± 187.5	275	249	56.9	0.84	0.20	0.81
Mean diameter, μm	43 ± 11.5	66	73	13.3	0.89	0.18	0.70
Cells ≤ 50 μm, % of total cells	72 ± 18.5	27	27	8.2	0.86	0.10	0.84
Cells > 50 ≤ 100 μm, % of total cells	26 ± 13.6	67	53	10.9	0.99	0.71	0.26
Cells > 100 μm, % of total cells	2 ± 9.3	7	20	17.1	0.94	0.29	0.52
Subcutaneous depot							
No. of steers	2	3	3				
Cell number/mm^2	181 ± 56.9	181	184	56.8	0.84	0.20	0.81
Mean diameter, μm	91 ± 13.3	91	88	13.3	0.89	0.18	0.70
Cells ≤ 50 μm, % of total	13 ± 8.2	13	10	8.2	0.86	0.10	0.84
Cells > 50 ≤ 100 μm, % of total cells	67 ± 10.9	49	62	10.9	0.99	0.71	0.52
Cells > 100 μm, % of total cells	7 ± 17.1	38	28	17.1	0.94	0.29	0.52

[1]Measured in hematoxylin + eosin stained adipose tissue slides (>1.5 mm^2 per sample).
[2]Restricted = no supplemental vitamin A, and control = supplemented with 2,200 IU of vitamin A/kg of DM.
[3]SEM of resticted and control diets.
[4]D = Diet (R vs. control), and F = Fat depot (intramuscular vs. subcutaneous).

slaughtered, those smaller adipocytes would have had the opportunity to fill with fat and become enlarged. A more intensive sampling schedule or a more sensitive laboratory technique to detect changes in cellularity may be required to evaluate the effect of vitamin A restriction on adipocyte hyperplasia.

Schoonmaker et al. (2004) previously reported that when Holstein steers were fed a high concentrate diet for 145 and 334 d, the i.m. fat depot presented a bi- and triphasic growth pattern, respectively. Based on the cell-size distribution of i.m. adipocytes in this experiment on d 1, 112, and 243 (Figure 1) it appeared that the i.m. depot had a monophasic pattern for vitamin A-restricted and control steers. Intramuscular adipocyte size distribution was similar between LR and SR steers, but the size of this fat depot was smaller in SR steers, as evidenced by the intramuscular EE content. Thus, the hyperplasia that presumably took place between d

Table 7. Effect of dietary vitamin A restriction on the cellularity of adipose tissue at slaughter (d 243) in Holstein steers

	Treatment				*P*-value[3]		
Item[1]	Long restriction	Short restriction	Control	SEM[2]	D	F	D × F
Intramuscular depot							
No. of steers	16	17	18				
Cell number/mm^2	177	190	201	11.6	0.63	<0.01	0.47
Mean diameter, μm	83	79	80	3.2	0.96	<0.01	0.51
Cells ≤ 50 μm, % of total cells	15	21	18	2.0	0.43	<0.01	0.29
Cells > 50 ≤ 100 μm, % of total cells	60	54	58	2.9	0.22	<0.01	0.93
Cells > 100 μm, % of total cells	25	26	24	3.6	0.78	<0.01	0.76
Subcutaneous depot							
No. of steers	16	17	18				
Cell number/mm^2	101	94	98	11.6	0.63	<0.01	0.47
Mean diameter, μm	112	115	113	3.2	0.96	<0.01	0.51
Cells ≤ 50 μm, % of total cells	4	4	4	2.0	0.43	<0.01	0.29
Cells > 50 ≤ 100 μm, % of total cells	36	32	34	2.9	0.22	<0.01	0.93
Cells > 100 μm, % of total cells	59	64	62	3.6	0.78	<0.01	0.76

[1]Measured in hematoxylin + eosin stained adipose tissue slides (>1.5 mm^2 per sample).
[2]For n = 16.
[3]D = Diet (R vs. control), and F = Fat depot (intramuscular vs. subcutaneous).

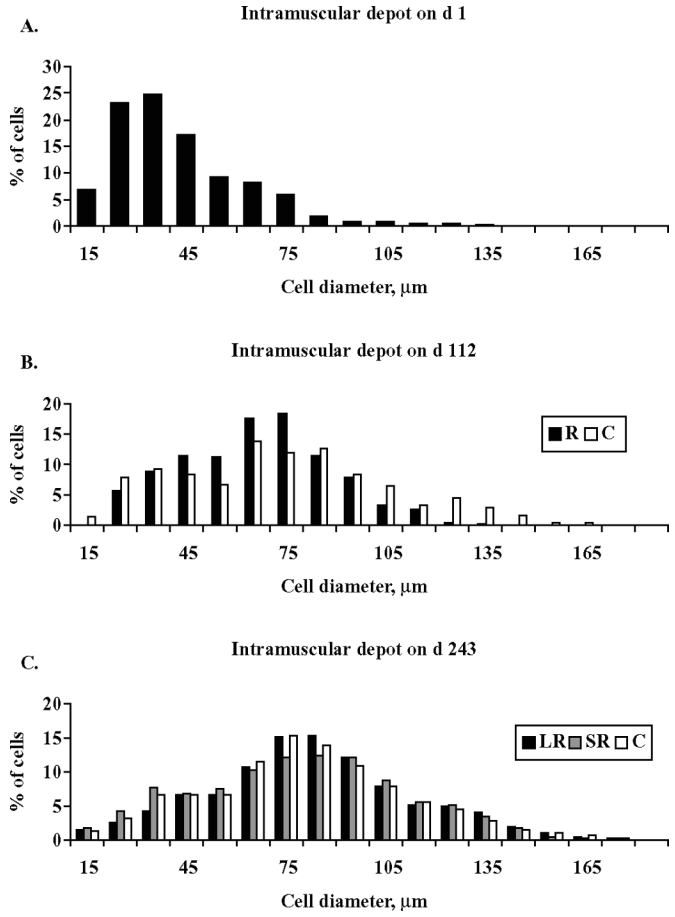

Figure 1. Effect of vitamin A restriction on intramuscular adipocyte cell diameter on A) d 1, B) d 112 (R = restricted, and C = control), and C) d 243 (LR = long restriction, SR = short restriction, and C = control).

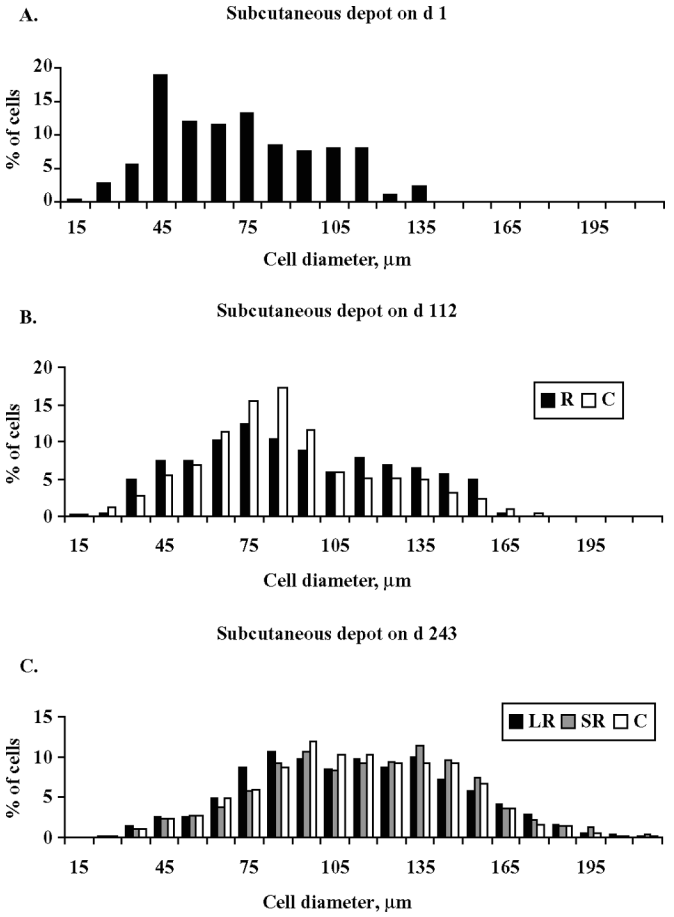

Figure 2. Effect of vitamin A restriction on subcutaneous adipocyte cell diameter on A) d 1, B) d 112 (R = restricted, and C = control), and C) d 243 (LR = long restriction, SR = short restriction, and C = control).

112 and 243 in the LR group may not have occurred in the SR group. It can be speculated that to stimulate i.m. fat deposition, vitamin A restriction needs to be longer than 131 d (duration of SR) or that it must occur early in the finishing period to allow the differentiation of new fat cells and their consequent enlargement as they fill with fatty acids, or both. Further research on this area is warranted.

The size of the s.c. depot was similar among treatments, based on the fat content of the edible carcass, depth of backfat, and USDA YG (all $P > 0.10$). Thus, it appears that vitamin A restriction does not affect s.c. fat deposition. The presumed mechanism of action of vitamin A restriction on i.m. fat deposition is the stimulation of adipocyte differentiation (Pyatt and Berger, 2005). Because the s.c. depot does not undergo extensive hyperplasia at the time steers are typically placed on finishing rations (Cianzio et al., 1985), vitamin A restriction would have no effect on this depot, as evidenced in this experiment.

Subcutaneous adipocytes were larger than i.m. adipocytes across all treatments on d 1, 112, and 243 (all $P < 0.01$). The s.c. adipocyte size distribution on d 1, 112, and 243 (Figure 2) indicates that the most frequent size of adipocytes was greater as days on feed increased ($P < 0.01$). It was concluded that cell enlargement rather than cell proliferation plays a greater role in the growth of this depot. Subcutaneous adipocyte size distribution did not appear to be affected by vitamin A restriction on d 112 or 243. The proportion of cells with a mean diameter of 95 to 115 μm on d 112 was similar ($P = 0.99$) between vitamin A-restricted and control steers. It has been suggested that when adipocytes reach a certain size, a new wave of cells emerges to continue the fat depot development (Hood and Allen, 1973; Robelin, 1986). The specific size at which a new wave of adipocytes emerged is still uncertain, but Schoonmaker et al. (2004) reported that size to be greater than 90 μm for the s.c. depot. Thus, conversely to what happened in the i.m. depot on d 112, the proportion of cells reaching the threshold size at which a new proliferative wave of cells emerge was not affected by vitamin A restriction.

Short and long vitamin A restriction reduced ($P < 0.01$) serum retinol content by d 243 (Table 8). Changes

Table 8. Effect of dietary vitamin A restriction on the serum retinol content of Holstein steers

	Treatment				
Item	Long restriction	Short restriction	Control	SEM[1]	*P*-value
d 1					
No. of steers	21	20	19		
Retinol, μg/dL	26.8	27.4	29.4	0.18	0.57
d 112					
No. of steers	20	19	18		
Retinol, μg/dL	27.6[x]	41.7[y]	37.6[y]	0.16	<0.01
d 243					
No. of steers	16	17	18		
Retinol, μg/dL	21.2[x]	25.2[x]	36.9[y]	0.18	<0.01

[x,y]Within a row, means without a common superscript letter differ, $^{P} < 0.05$.
[1]For n = 19 on d 1, n = 18 on d 112, and n = 16 on d 243.

in serum retinol reflected the apparent depletion of retinol stores in the liver (Tables 9 and 10) of LR and SR steers by d 243 ($P < 0.01$). Serum retinol levels on d 112 were greater than on d 1 for all treatments. Serum retinol levels are kept relatively constant to maintain bodily functions. Liposoluble vitamins such as retinol can be stored in the liver and released to the bloodstream to meet animal requirements (Blaner and Olson, 1994). To detect reductions in serum retinol, liver stores need to be depleted. Thus, changes in the site of fat deposition in response to dietary vitamin A restriction likely would first require liver retinol stores to be depleted. Little is known about liver retinol depletion kinetics in dietary vitamin A-restricted feedlot steers. An 86% reduction in liver retinol concentrations was observed on d 112 in vitamin A-restricted steers, whereas control steers had 62% reduced liver retinol concentrations on d 112 ($P < 0.10$). The severity of the observed reduction in both groups suggests that initial liver retinol stores were unusually high for Holstein steers. The rearing conditions of these animals before the experiment was unknown, although it is possible that these animals consumed fresh green forage with ample provitamin A carotenoid content or dairy calf starter rations in which vitamin A supplementation was considerably greater than in our control ration. The amount of hepatic vitamin A stores may play a crucial role in the effectiveness of dietary vitamin A restriction during the finishing period to stimulate i.m. fat deposition. To stimulate i.m. adipocyte differentiation and ultimately marbling, it may not be sufficient to deplete hepatic retinol stores at the end of the finishing period as evidenced by the SR treatment. Apparently, hepatic vitamin A stores need to be quickly depleted and maintained low for a yet undetermined period of time to affect the i.m. fat depot. Further research is advised to determine the duration of vitamin A restriction required to observe changes in i.m. fat deposition.

Serum retinol on d 243 was lower ($P < 0.01$) in LR and SR compared with control steers (Table 8). Observed concentrations in LR and SR are not considered to be deficient (Weiss, 1998). This supports our assessment that it is possible to affect the site of fat deposition without compromising animal performance and health.

The adipose tissue is the second largest depot in which retinol is stored in the body (Tsutsumi et al., 1992). The amount of vitamin A stored in s.c. fat is important because it may be readily available to adipocytes in that depot or in other depots of the body (i.e., intramuscular) via the bloodstream. Subcutaneous fat

Table 9. Effect of dietary vitamin A restriction for 112 d on the retinol content of liver and subcutaneous fat in Holstein steers

	Time on feed, d				
		112			
Item	0	Restricted[1]	Control[1]	SEM[2]	*P*-value[2]
No. of steers	3	3	3		
Liver retinol, μg/g	105 ±	15	40	7.6	0.08
s.c. fat retinol, μg/g	0.8 ± 0.26	0.5	0.7	0.12	0.33

[1]Restricted = no supplemental vitamin A, and control = supplemented with 2,200 IU of vitamin A/kg of DM.
[2]SEM and *P*-value of restricted vs. control diet.

Table 10. Effect of dietary vitamin A restriction on the retinol content of liver and subcutaneous fat at slaughter (d 243) in Holstein steers

	Treatment				
Item	Long restriction	Short restriction	Control	SEM[1]	*P*-value
No. of steers	16	15	16		
Liver retinol, μg/g	6.1[x]	6.5[x]	44.7[y]	4.8	<0.01
s.c. fat retinol, μg/g	0.47	0.73	0.56	0.12	0.33

[x,y]Within a row, means without a common superscript letter differ, $P < 0.05$.
[1]For n = 15.

retinol was not affected by vitamin A restriction on d 112 or 243 ($P > 0.10$), suggesting that fat plays a passive role in the modulation of body vitamin A status. It remains unknown whether there are differences in the retinol content between adipose depots and if retinol stored in fat may act as a local differentiation repressor.

Table 11. Effect of dietary vitamin A restriction for 112 d on fatty acid composition of subcutaneous fat

	Time on feed, d				
		112			
Item	0	Restricted	Control[1]	SEM[2]	*P*-value[2]
No. of steers	2	3	2		
	g/100 g of total fatty acids				
Fatty acid					
14:0	3.28 ± 0.061	4.2	3.6	0.14	0.08
14:1	0.56 ± 0.040	0.9	0.6	0.11	0.16
15:0	0.73 ± 0.015	0.76	0.57	0.068	0.18
16:0	26.1 ± 0.26	28	27	1.4	0.54
16:1	3.6 ± 0.32	4.4	3.3	0.32	0.13
17:0	2.2 ± 0.16	2.0	1.8	0.30	0.75
17:1	1.27 ± 0.035	1.3	1.1	0.27	0.69
18:0	15.5 ± 0.40	13	18	1.9	0.19
18:1, total *trans*[3,4]	7 ± 1.4	5	5	1.1	0.80
18:1, *c*-9	33 ± 0.33	34	36	1.5	0.64
18:1, *c*-11	0.8 ± 0.78	1.6	0.8	0.27	0.14
18:2	4.3 ± 0.51	2.8	2.5	0.27	0.45
18:2, CLA *c*9*t*11	0.1 ± 0.13	0.25	0.24	0.024	0.76
18:2, CLA *t*10*c*12	0.02 ± 0.019	0.009	0.022	0.0082	0.51
18:2, other CLA	0.03 ± 0.026	0.06	0.05	0.022	0.79
18:3n-3	0.04 ± 0.037	0.03	0.03	0.022	0.92
20:0	0.05 ± 0.051	0.03	0.04	0.023	0.92
20:1	0.2 ± 0.15	0.20	0.21	0.044	0.87
20:2	0.03 ± 0.026	0.02	0.03	0.020	0.62
20:3n-6	0.04 ± 0.044	0.03	0.04	0.032	0.81
20:3n-3	0.02 ± 0.019	0.03	0.03	0.026	0.95
22:6	0.09 ± 0.086	0.04	0.06	0.043	0.84
24:1	0.09 ± 0.087	0.19	0.06	0.042	0.15
Total CLA	0.2 ± 0.17	0.31	0.30	0.053	0.89
16:1/16:0	12.0 ± 0.84	14	11	1.4	0.38
Desaturase index[5]	46 ± 0.49	48	46	2.5	0.69
MUFA[6]	40 ± 1.3	43	42	2.0	0.68
PUFA[7]	5 ± 0.30	3.0	2.7	0.29	0.59
SFA[8]	48 ± 0.30	48	50	2.8	0.65

[1]Restricted = no supplemental vitamin A, and control = supplemented with 2,200 IU of vitamin A/kg of DM.
[2]SEM and *P*-value of restricted vs. control diet.
[3]Includes 18:1, *t*6, *t*7, *t*, *t*9, *t*10, *t*11, and *t*12.
[4]The presence of 18:1, *t*10 was considerably greater than that of the other 18:1 *trans* isomers.
[5](16:1 + 18:1, *c*-9 + 18:1, *c*-11/(14:0 + 16:0 + 18:0 + 16:1 + 18:1, *c*-9 + 18:1, *c*- 11).
[6]14:1 + 16:1 + 18:1, *t*-10 + 18:1, *t*-11 + 18;1, *t*-other + 18:1, *c*-9 + 18:1, *c*-11 + 20:1 + 22:1 + 24:1.
[7]18:2 + 18:3, n-6 + 18:3, n-3 + 20:2 + 20:3, n-6 + 20:3, n-3 + 20:4 + 20:5 + 22:2 + 22:6.
[8]14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 20:0 + 22:0 + 24:0.

Table 12. Effect of dietary vitamin A restriction on fatty acid composition of subcutaneous fat at slaughter (d 243) in Holstein steers

Item	Treatment			SEM[1]	*P*-value
	Long restriction	Short restriction	Control		
No. of steers	16	17	18		
	g/100 g of total fatty acids				
Fatty acid					
14:0	3.7	4.1	4.1	0.21	0.35
14:1	1.13	1.22	1.17	0.096	0.80
15:0	0.61	0.66	0.66	0.037	0.48
16:0	28.6	29.6	29.8	0.71	0.46
16:1	4.9	4.7	4.8	0.31	0.97
17:0	1.62	1.73	1.65	0.085	0.67
17:1	1.22	1.19	1.17	0.052	0.82
18:0	11.1	11.0	11.6	0.54	0.74
18:1, total *trans*[2,3]	3.6	4.6	3.6	0.31	0.06
18:1, *c*-9	38	36	37	1.1	0.33
18:1, *c*-11	1.2	1.1	0.9	0.20	0.62
18:2	1.9	2.1	1.9	0.11	0.46
18:2, CLA *c*9*t*11	0.17	0.18	0.17	0.016	0.71
18:2, CLA *t*10*c*12	0.011	0.009	0.011	0.0031	0.81
18:2, other CLA	0.038	0.061	0.060	0.0079	0.45
18:3n-6	0.001	0.011	0.000	0.0018	0.07
18:3n-3	0.09	0.08	0.10	0.017	0.69
20:0	0.055	0.052	0.057	0.0061	0.58
20:1	0.25	0.23	0.20	0.016	0.08
20:2	0.07	0.07	0.06	0.016	0.83
20:3n-6	0.068	0.071	0.071	0.0078	0.98
20:3n-3	0.040	0.048	0.041	0.0071	0.51
20:4	0.03	0.05	0.05	0.026	0.75
20:5	0.000	0.001	0.001	0.0017	0.84
22:0	0.008	0.007	0.008	0.0067	0.78
22:1	0.00	0.01	0.00	0.004	0.16
22:2	0.01	0.03	0.02	0.011	0.63
22:6	0.13	0.13	0.12	0.024	0.91
24:1	0.27	0.24	0.28	0.040	0.73
Total CLA	0.22	0.25	0.24	0.021	0.59
16:1/16:0	14.4	13.8	14.0	0.81	0.86
Desaturase index[4]	51	49	48	1.3	0.44
MUFA[5]	48	45	46	1.3	0.38
PUFA[6]	2.5	2.6	2.4	0.12	0.41
SFA[7]	46	47	48	1.2	0.46

[1]For n = 16.
[2]Includes 18:1 *t*6–8, *t*9, *t*10, *t*11, and *t*12.
[3]The presence of 18:1, *t*10 was considerably greater than that of the other 18:1 *trans* isomers.
[4](16:1 + 18:1, *c*-9 + 18:1, *c*-11/(14:0 + 16:0 + 18:0 + 16:1 + 18:1, *c*-9 + 18:1, *c*- 11).
[5]14:1 + 16:1 + 18:1, *t*-10 + 18:1, *t*-11 + 18;1, *t*-other + 18:1, *c*-9 + 18:1, *c*-11 + 20:1 + 22:1 + 24:1.
[6]18:2 + 18:3, n-6 + 18:3, n-3 + 20:2 + 20:3, n-6 + 20:3, n-3 + 20:4 + 20:5 + 22:2 + 22:6.
[7]14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 20:0 + 22:0 + 24:0.

The effects of duration of vitamin A restriction on s.c. fatty acid proportions are presented in Tables 11 and 12. Vitamin A restriction for 112 (R = 0.31 vs. control = 0.30% of total fatty acids, $P = 0.89$), 131, or 243 d (LR = 0.22, SR = 0.25, and control = 0.24%, $P = 0.59$) did not affect beef s.c. fat total CLA proportion. It was hypothesized that restricting dietary vitamin A would increase SCD activity and therefore further stimulate the conversion of vaccenic to rumenic acid in beef tissues. Our previous results were not clear in this area. Feeding low-vitamin A diets for 168 d to Angus-based steers tended to decrease CLA proportion in s.c. fat but not in LM (Gorocica-Buenfil et al., 2007). The activity of SCD enzyme has been reported to be reduced by retinol and its precursor β-carotene (Alam and Alam, 1985; Siebert and Zurk, 2004). Increasing the duration of vitamin A restriction from our previous experiment was expected to reduce the retinol inhibition of SCD allowing a greater accumulation of CLA in beef tissues. Our results do not support this hypothesis. Genetic differences between dairy and beef breeds on SCD activity and regulation may have been involved in the lack of agreement between this and our previous experiment. Additionally, in this experiment no supplemental lino-

leic acid source was added to the diet. It is possible that the effectiveness of dietary vitamin A restriction to increase proportion of CLA in s.c. fat may have been compromised by a shortage of linoleic acid supply in the rumen, because this fatty acid is the precursor for CLA synthesis. It is also possible that vitamin A restriction could affect SCD activity without affecting beef CLA content because this enzyme catalyzes numerous metabolic reactions besides CLA synthesis. Desaturase activity index is an indirect indicator that measures the SCD activity based on its substrates to products ratio (Corl et al., 2001; Smith et al., 2002). Dietary vitamin A restriction for 112 (R = 47.7% vs. control = 45.9%, $P = 0.69$), 131, or 243 d (LR = 51.2%, SR = 49.2%, and control = 48.9%, $P = 0.44$) did not affect beef s.c. desaturase activity index. Conflicting data are available in the literature regarding the effects of retinol on SCD. Some authors suggest that retinol inhibits SCD activity (Alam and Alam, 1985; Siebert et al., 2003), whereas others report that retinol increases SCD transcription (Daniel et al., 2004). Lucchi et al. (2005) suggested that SCD activity was stimulated by retinol, reporting increased CLA levels in blood in human patients with high-vitamin A plasma levels. In the present experiment greater levels of serum retinol in control steers did not affect s.c. fat CLA. Genetic differences between Holstein and Angus-based steers may exist regarding the effects of vitamin A restriction on SCD activity. Because feeding low-vitamin A diets seems to be an effective strategy to increase i.m. fat deposition, studying the effect of dietary vitamin A restriction on beef fatty acid composition is warranted.

In conclusion, restricting vitamin A intake for 243 d increased i.m. fat percentage without affecting s.c. or visceral fat deposition, feedlot performance, or carcass weight. Restricting vitamin A intake for 131 d at the end of the finishing period appears to be insufficient to affect the site of fat deposition in Holstein steers. Regardless of duration, vitamin A restriction did not appear to affect s.c. fat CLA content and had little effect on the concentration of other fatty acids.

LITERATURE CITED

Alam, S. Q., and B. S. Alam. 1985. Microsomal fatty acid desaturase activities in vitamin A-deficient rat liver. Biochim. Biophys. Acta 833:175–177.

AOAC. 1996. Official Methods of Analysis. 16th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Blaner, W. S., and J. A. Olson. 1994. Retinol and retinoic acid metabolism. Pages 5–178 in The Retinoids: Biology, Chemistry and Medicine. 2nd ed. M. B. Sporn, A. B. Roberts, and D. S. Goodman, ed. Raven Press, Ltd., New York, NY.

Ching, S., D. C. Mahan, T. G. Wiseman, and N. D. Fastinger. 2002. Evaluating the antioxidant status of weanling pigs fed dietary vitamins A and E. J. Anim. Sci. 80:2396–2401.

Cianzio, D. S., D. G. Topel, G. B. Whitehurst, D. C. Beitz, and H. L. Self. 1985. Adipose tissue growth cellularity: Changes in bovine adipocyte size and number. J. Anim. Sci. 60:970–976.

Corl, B. A., L. H. Baumgard, D. A. Dwyer, J. M. Griinari, B. S. Phillips, and D. E. Bauman. 2001. The role of Δ^9–desaturase in the production of *cis*-9, *trans*-11 CLA. J. Nutr. Biochem. 12:622–630.

Culling, C. F. A. 1974. Handbook of Histopathological and Histochemical Techniques. 3rd ed. Butterworth, London, UK.

Daniel, Z. C. T. R., A. M. Salter, and P. J. Buttery. 2004. Vitamin A regulation of stearoyl-CoA desaturase mRNA levels and fatty acid composition in sheep tissues. Anim. Sci. 78:237–243.

Eaton, H. D., J. E. Rousseau, Jr., R. C. Hall, Jr., H. I. Frier, and J. J. Lucas. 1972. Reevaluation of the minimum vitamin A requirement of Holstein male calves based upon elevated cerebrospinal fluid pressure. J. Dairy Sci. 55:232–237.

Galyean, M. L., and J. F. Gleghorn. 2002. Summary of the 2000 Texas Tech University consulting nutritionist survey. In Proc. Plain Nutr. Counc. Conf., San Antonio, TX. Texas A&M Research and Extension Center, Amarillo.

Garrett, W. N., and N. Hinman. 1969. Re-evaluation of the relationship between carcass density and body composition of beef steers. J. Anim. Sci. 28:1–5.

Gorocica-Buenfil, M. A., F. L. Fluharty, C. K. Reynolds, and S. C. Loerch. 2007. Effect of dietary vitamin A concentration and roasted soybean inclusion on marbling, adipose cellularity and fatty acid composition of beef. J. Anim. Sci. 85:2230–2242.

Hankins, O. G., and P. E. Howe. 1946. Estimations of the composition of beef carcasses and cuts. USDA Tech. Bull. 926. Washington, DC.

Hood, R. L., and C. E. Allen. 1973. Cellularity of bovine adipose tissue. J. Lipid Res. 14:605–610.

Kramer, J. K. G., V. Fellner, M. E. R. Dugan, F. D. Sauer, M. M. Mossaba, and M. P. Yurawecz. 1997. Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total *trans* fatty acids. Lipids 32:1219–1228.

Kumar, M. V., G. D. Sunvold, and P. J. Scarpace. 1999. Dietary vitamin A supplementation in rats: Suppression of leptin and induction of UCP-1 mRNA. J. Lipid Res. 40:824–829.

Lucchi, L., S. Banni, A. Iannone, M. P. Melis, G. Carta, E. Murru, L. Cordeddu, L. Stipo, S. Uggeri, V. Gatti, V. Malaguti, and A. Albertazzi. 2005. Changes in conjugated linoleic acid and palmitoleic acid are correlated to retinol levels in chronic renal failure in both hemodialysis and conservative treatment patients. Artif. Organs 29:413–418.

NRC. 1976. Nutrient Requirements of Beef Cattle. 5th rev. ed. Natl. Acad. Press, Washington, DC.

NRC. 1996. Nutrient Requirements of Beef Cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Perry, T. W., W. M. Beeson, W. H. Smith, R. B. Harrington, and M. T. Mohler. 1968. Interrelationships among vitamins A, E, and K when added to the rations of fattening beef cattle. J. Anim. Sci. 27:190–194.

Perry, T. W., W. M. Beeson, W. H. Smith, and M. T. Mohler. 1965. Value of supplemental vitamin A for fattening beef cattle on pasture. J. Anim. Sci. 25:814–816.

Pyatt, N. A., and L. L. Berger. 2005. Review: Potential effects of vitamins A and D on marbling deposition in beef cattle. Prof. Anim. Sci. 21:174–181.

Robelin, J. 1986. Growth of adipose tissues in cattle; partitioning between depots, chemical composition and cellularity. A review. Livest. Prod. Sci. 14:349–364.

Sato, M., A. Hiragun, and H. Matsui. 1980. Preadipocytes possess cellular retinoid binding proteins and their differentiation is inhibited by retinoids. Biochem. Biophys. Res. Commun. 95:1839–1845.

Schoonmaker, J. P., F. L. Fluharty, and S. C. Loerch. 2004. Effect of source and amount of energy and rate of growth in the growing phase on adipocyte cellularity and lipogenic enzyme activity in the intramuscular and subcutaneous fat depots of Holstein steers. J. Anim. Sci. 82:137–148.

Siebert, B. D., W. S. Pitchford, Z. A. Kruk, H. Kuchel, M. P. B. Deland, and C. D. K. Bottema. 2003. Differences in Δ^9 desaturase activity between Jersey- and Limousin-sired cattle. Lipids 38:539–543.

Siebert, B. D., and Z. A. Zurk. 2004. Beta-carotene and oxidative desaturation of fatty acids: A plausible explanation of the con-

flicting responses of coronary heart disease to beta-carotene? Med. Hypoth. 62:950–953.

Smith, S. B., T. S. Hively, G. M. Cortese, J. J. Han, K. Y. Chung, P. Castañeda, C. D. Gilbert, V. L. Adams, and H. J. Mersmann. 2002. Conjugated linoleic acid depresses the Δ^9 desaturase index and stearoyl coenzyme A desaturase enzyme activity in porcine subcutaneous adipose tissue. J. Anim. Sci. 80:2110–2115.

Tsutsumi, C., M. Okuno, L. Tannus, R. Piandetosi, M. Allan, D. S. Goodman, and W. S. Blaner. 1992. Retinoids and retinoid-binding protein expression in rat adipocytes. J. Biol. Chem. 267:1805–1810.

USDA. 1997. Standards for Grades of Carcass Beef. Agric. Marketing Service, USDA, Washington, DC.

USDA Agricultural Marketing Service. 2006. Comparison of certified beef programs. http://www.ams.usda.gov/lsg/certprog/speccomp.pdf Accessed Apr. 22, 2006.

USDA Market News Service. 2005. National weekly direct slaughter cattle—Premiums and Discounts. For the Week of: 10/17/2005. http://www.ams.usda.gov/mnreports/lm_ct155.txt Accessed Oct. 23, 2005.

Weiss, W. P. 1998. Requirements of fat-soluble vitamins for dairy cows: A review. J. Dairy Sci. 81:2493–2501.