Effects of Vitamins A, D₃ and E on Growth Performance and Carcass Characteristics, and on Lipolytic Responsiveness and Insulin Secretion in Japanese Black Steers

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Abstract To investigate the effects of restricted vitamins A, D₃ and E intake on feeding performance, carcass characteristics, and on metabolic and hormonal status, 7 Japanese Black steers (15.2±0.1 months of age) were used in an 11 months feeding trial. Steers had free access to a rice straw and a concentrate either supplemented with (+ VIT ; n=4) or without (- VIT ; n=3) 7,500IU vitamin A, 1,500IU vitamin D₃ and 7.5IU vitamin E per 1 kg of the concentrate until slaughtered at 26 months of age. Monthly blood samples were collected to determine plasma concentrations of retinol, metabolites, insulin and insulin-like growth factor-1. Intravenous challenges of nor-epinephrine (NE ; 0.01 mg/kg BW) and glucose (0.625 mM/kg BW) were administered separately at the age of 17, 21 and 25 months, to assess *in vivo* lipolytic responsiveness and insulin secretion. After 21 months of age, the - VIT steers had significantly lower plasma retinol levels. Body weights and body weight gain during the experiment tended to be higher in + VIT steers than in - VIT steers, whereas feed intake was not affected by supplemental vitamins. Vitamin supplementation did not affect any of carcass traits including lipid content in *M. longissimus thoracis*. Although a tendency for elevated plasma cholesterol in the - VIT steers was observed, other metabolite and hormone concentrations and the responses to NE and glucose challenge were not affected by the restriction of vitamins A, D₃ and E intake. The restricted vitamins A, D₃ and E intake in Japanese Black steers during the period from 15 to 26 months old appeared to be ineffective for improvement of marbling performance.

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Key words : Vitamins A, D₃, E, Feeding performance, Carcass characteristics, Beef steers, Insulin secretion

Current topic concerning feeding management of beef cattle in Japan is whether vitamin A deficiency can actually improve beef quality, especially in respect to marbling performance. Several workers^{24,36)} reported that blood retinol level was negatively related to marbling grade in Wagyu beef cattle. Inhibitory effect of retinol on the differentiation of preadipocyte to

adipocyte in ST 13 or 3 T 3-L 1 cell line has been reported^{18,33)}. Retinoic acid has also been shown to inhibit the differentiation of preadipocytes from newborn pigs in primary culture³⁴⁾. Antiadipogenic action of retinoid is thought to be mediated by specific nuclear receptor, which act as an ligand-dependent transcriptional regulator²⁰⁾.

Although several experiments have recently been performed to clarify the relationship between vitamin A status and meat quality of fattening cattle (Hodate, Proceedings of Japanese Society for Animal Nutrition and Metabolism, 39 : 157-171, 1995), information about the influences of vitamin A deprivation on feed intake, feed conversion efficiency, and fat deposition and its partitioning, and on endocrine and metabolic status of Wagyu fattening steers is limited. Until now, attention have been paid only on the association of vitamin A status and beef quality. However, the practical vitamin restricted feeding, which limits the use of vitamin A, seems to cut off vitamin premix containing vitamins A, D₃ and E from the fattening ration. Thus, the present study was conducted to investigate the effects of dietary supplementation of vitamins A, D₃ and E to a conventional concentrate/rice straw diet, which did not include these vitamins as a supplement, on live animal performance and car-

cass characteristics of individually fed Japanese Black steers. The influence of the restricted vitamins A, D₃ and E intake on plasma concentrations of metabolite, insulin and insulin-like growth factor-1 (IGF-1), and on responses to nor-epinephrine (NE) and glucose were also determined during the fattening period.

Materials and Methods

Animals and design

Seven Japanese Black steers (15.2 ± 0.1 months of age) were used in an 11 months feeding trial. Steers were housed in a concrete-floored pen equipped with individual discriminating door feeder, and had free access to a concentrate and chopped rice straw described in Table 1 throughout the experimental period. Water and trace mineral salt were freely available. Steers were assigned to one of two treatments, balanced for their sire bull, body weight (BW) and pre-experimental management.

Table 1. Chemical composition of experimental feeds

Item	Concentrate	Rice straw
Ingredient ¹ (% as fed basis)		
Barley, Corn and Milo	71	—
Soybean meal	15	—
Wheat bran	12	—
Calcium carbonate and NaCl	2	—
Chemical composition ² (DM basis)		
Dry matter (%)	87.2	87.8
Crude protein (%)	17.3	5.8
Ether extract (%)	3.4	1.3
Neutral detergent fiber (%)	15.4	70.3
Acid detergent fiber (%)	5.4	39.9
Organic cell wall ³ (%)	16.9	73.1
Organic a fraction ³ (%)	2.3	8.5
Organic b fraction ³ (%)	14.6	64.6
Metabolizable energy ⁴ (Mcal/kg)	3.09	1.28
Gross energy (Mcal/kg)	4.42	4.05

¹ Commercially formulated to contain 13.0% of digestible crude protein and 71.5% of total digestible nutrients (as fed basis).

² Means of 10 samples for concentrate, and of 5 samples for rice straw.

³ Analyzed according to the method of Abe¹¹.

⁴ Estimated from Terada *et al.*¹⁰.

Treatments were a concentrate either not supplemented (–VIT) or supplemented with vitamins A, D₃ and E (+VIT). Three steers were assigned to –VIT group and 4 steers were assigned to +VIT group. A commercial formula concentrate feed for fattening cattle, which was formulated not to contain supplemental vitamins, was used as the –VIT concentrate. Vitamin supplemented concentrate (+VIT concentrate) was prepared every month, to contain 7,500 IU of vitamin A, 1,500 IU of vitamin D₃ and 7.5 IU of vitamin E (dl- α -tocopheryl acetate) per 1 kg fresh matter of the concentrate identical to the –VIT concentrate. Chemical analysis of feeds were done according to the method of Abe¹⁾ and AOAC²⁾. The metabolizable energy (ME) content of the feed was predicted from the chemical composition according to Terada *et al.*³⁵⁾. Individual feed intake and BW were recorded weekly during the whole experimental period.

Slaughter and carcass measurements

At 26 months of age, steers were slaughtered at an experimental abattoir after 48 hours feed deprivation and 24 hours restriction of water intake. Carcass and all offal components except for blood and the contents of digestive tracts were measured to calculate empty BW. Viscera and omental, mesenteric, kidney and heart fat were separated and weighed. After an overnight chill at 0°C, longissimus area and thickness of subcutaneous fat, intermuscular fat and belly were measured at 6–7th rib cross section according to Japan Meat Grading Association¹⁷⁾. Then the left side of each carcass was dissected and separated into the bone, lean, subcutaneous fat, intermuscular fat, abdominal surface fat and others including tendon and fascia. Sample of *M. longissimus thoracis* was obtained at the 7th rib portion and minced by passing through a meat chopper at least 3 times. The minced meat sample was stored at –30°C until analyzed for chemical composition by the method of AOAC²⁾. Moisture was determined by weight loss after

drying at 100°C for 16 hours, and mineral content by ashing at 600°C for 3 hours. Nitrogen was determined with the Kjeldahl method and multiplied by 6.25 to calculate crude protein content. Crude fat was determined by Soxhlet extraction with diethyl ether for 16 hours.

Blood collection and metabolic challenges

Blood samples were collected monthly via jugular venipuncture at 08:00. Sodium heparin was used as an anticoagulant for most of blood samples except for blood samples for the analyses of non-esterified fatty acid (NEFA) and retinol. EDTA-2K was used as an anticoagulant to determine plasma concentrations of NEFA and retinol in blood samples. The blood sample was immediately chilled in a cooled container (0–7°C). Plasma was separated by centrifugation (1,710×g for 15 minutes) at 4°C and stored at –30°C for subsequent hormone and metabolite analyses. Plasma concentrations of glucose, urea nitrogen, cholesterol, NEFA and insulin were determined in monthly plasma samples. Plasma retinol concentration was determined in the samples collected at the age of 15, 17, 19, 21, 23, 25 and 26 months. Plasma IGF-1 concentration was determined in the samples collected at the age of 15, 17, 19, 21, 23 and 25 months.

At the age of 17, 21 and 25 months, steers were subjected to metabolic challenges consisted of 1 µg/kg BW of thyrotropin releasing hormone (TRH) (200 µg/ml, Peptide institute, inc., Osaka, Japan), 0.01 mg/kg BW of NE (1 mg/ml, nor-adrenalin, Sankyo, Tokyo, Japan) and 0.625 mM/kg BW of glucose (500 mg/ml). Steers were fitted with an indwelling jugular catheter on the day prior to TRH challenge. Catheters were maintained patent with a sterile 3.8% trisodium citrate solution lock. Catheters were flushed with 3–5 ml of the trisodium citrate solution after each challenge injection, and were filled with 1–2 ml of the trisodium citrate solution subsequent to each blood sampling. With at least 1 day separated to the

TRH challenge, NE challenge was injected at 10:00 via the jugular catheter. Blood samples were taken from the catheter at -30, -15, 0, 5, 10, 20, 30, 45, 60, 90 and 120 minutes relative to the NE injection. Blood sample was treated as described for monthly blood collection. Plasma was collected and stored at -30°C until analyzed for concentrations of glucose and NEFA. After NE challenge, another indwelling catheter for glucose infusion was inserted into the lateral side of jugular vein. With at least 1 day separated to the NE challenge, glucose challenge was carried out. Glucose solution was infused via one jugular catheter for 15 minutes (10:00-10:15), at a constant speed using peristaltic pump. The glucose solution was sterilized by passage through a 0.45 µm cellulose acetate sterile disk filter. Blood samples were taken from the opposite jugular catheter at -30, -15, 0, 5, 10, 15, 20, 25, 35, 45, 60, 75, 105 and 135 minutes relative to the initiation of glucose infusion. Blood sample was treated as described for monthly blood collection. Plasma was collected and stored at -30°C until analyzed for concentrations of glucose and insulin.

Assay of metabolites and hormones

Plasma glucose concentration was determined by the glucose oxidase-peroxidase method using an automatic glucose analyzer (GLU-11, TOA electronics Co. Ltd., Tokyo, Japan). Commercial kits (Wako Pure Chemicals, Osaka, Japan) were used for the analysis of urea nitrogen by the diacetylmonoxime method (urea nitrogen-test), cholesterol by the cholesterol oxidase-phenol method (cholesterol C-test) and NEFA by the acyl CoA synthetase-acyl CoA oxidase method (NEFA C-test).

Plasma insulin concentration was determined by radioimmunoassay (RIA) using an anti-bovine insulin guinea pig serum (Code No. 270400, SEIKAGAKU-KOGYO, Tokyo, Japan), according to the method of Sasaki and Takahashi³¹. Bovine insulin (Cat. No. 890-

8125 IG, 28.2 IU/mg, GIBCO BRL, Gaithersburg, MD, USA) was used as the reference standard, and ¹²⁵I-labelled human insulin (Code No. IM 166, Amersham Life Sci. Co. Ltd., Tokyo, Japan) was used as tracer. Intra- and interassay coefficient of variation (CV) were 6.23% (n=12) and 3.52% for 4 assays, respectively. IGF-1 concentration was determined by RIA using commercially available kit (Code No. IM 1721, Amersham Life Sci. Co. Ltd., Tokyo, Japan) validated for IGF-1 in bovine plasma²³. Recombinant human IGF-1 (TOYOBO Co. Ltd., Osaka, Japan) was used for the reference standard. Plasma was extracted with acid ethanol before assay⁹. All samples were run in a single RIA with an intra-assay CV of 5.2% (n=6). Plasma concentration of retinol was determined using HPLC method described by Driskell *et al.*¹⁰. Quantification of retinol was performed by The Chemo-Sero-Therapeutic Research Institute "KAKETSUKEN".

Calculations and statistical analysis

Metabolite and hormone responses to the challenges were calculated as total area under the response curve (AUC), except for NEFA response to the NE challenge. For the NE challenges, peak plasma concentrations of NEFA occurred 10-20 minutes after NE injection and returned to base line by 60-90 minutes for all steers at all ages, but some increases in plasma NEFA were observed after 60-90 minutes postchallenge in certain animals at 17 and 21 months of age. Thus, the NEFA area was calculated from the time of challenge to 30 minutes postchallenge, to minimize the contribution of counterregulatory effects. Response areas were corrected for differences in baseline concentration (mean of concentrations at -30, -15 and 0 minutes from the time of challenge administration). The rate of clearance of plasma glucose after the glucose challenge was determined from glucose concentrations in the initial declining phase of the response (0-30 minutes postchallenge). This was expressed as the fractional rate of change and

was determined from the slope of the natural logarithm of glucose concentrations plotted versus time.

All data were analyzed using the GLM procedure of SAS⁹⁰. Performance and carcass data were analyzed by analysis of variance at a completely random design. These data were also analyzed using initial BW, average BW and carcass weight as a covariate when appropriate, and revealed that the significance of the treatment effects for any variables of performance and carcass characteristics was not altered by these covariates. Therefore, these covariates were left out of the statistical model. Weekly measurements of BW and ME intake per unit of metabolic body size (MBS; kg BW^{0.75}), plasma hormone and metabolite concentrations, and responses to metabolic challenges were analyzed for split plot in time design with repeated measurements as described by Gill and Hafs¹³. If the treatment effect or treatment $\times$ time interaction had $P < 0.10$, the treatment means within each period were compared by least significant difference procedure. If the time effect had $P < 0.10$, the means across treatments over time were compared by Duncan's multiple range test. Significance was declared when the probability of no difference between the means was $P < 0.05$. Trends and tendency toward significance were assumed if $0.05 \leq P < 0.10$.

Results and Discussion

The overall performance of the cattle is presented in Table 2, and weekly means are shown in Fig. 1. Average daily gain for the +VIT steers was 13% greater than that for -VIT steers, although the difference was not significant ($P=0.14$). Total BW gain for the whole experimental period (data not shown) tended to be greater ($P=0.09$) in the +VIT steers than in -VIT steers. During the experiment, the +VIT steers consistently showed higher ($P=0.09$) BW than the -VIT steers (Fig. 1). An improvement of growth rate by supplemental vitamins agreed well with the previous reports^{6,24,28}. Dry matter and ME intake was not affected by the restriction of vitamins A, D₃ and E intake. It has been reported that feed intake of beef cattle was not changed by supplemental vitamin A or E alone or both A and E in combination^{6,12,28}. Metabolizable energy intake per unit of MBS for both groups decreased ($P < 0.01$) during the experiment. The steers for both groups consumed extremely low ratio of rice straw DM in the consumed total feed DM (overall mean across treatment groups: $5.4 \pm 0.6\%$) which was voluntarily ingested. However, no clinical symptom of any metabolic disorder such as bloat or acidosis was observed in any steers throughout the experiment.

Table 2. Performance data of the steers fed concentrate either supplemented (+VIT) or not supplemented (-VIT) with vitamins A, D₃ and E¹

Item	Treatment		P-value
	+ VIT	- VIT	
Number of animals	4	3	
Initial body weight (kg)	392 $\pm$ 23	369 $\pm$ 13	0.46
Final body weight (kg)	673 $\pm$ 30	617 $\pm$ 22	0.22
Daily gain (kg/d)	0.86 $\pm$ 0.04	0.76 $\pm$ 0.04	0.14
Dry matter intake (kg/d)	8.18 $\pm$ 0.40	7.90 $\pm$ 0.19	0.60
Metabolizable energy intake (Mcal/d)	24.54 $\pm$ 1.23	23.56 $\pm$ 0.46	0.54
Dry matter intake/body weight gain	9.54 $\pm$ 0.37	10.52 $\pm$ 0.63	0.21

¹ Means $\pm$ SEM

Fig. 1. Metabolizable energy (ME) intake (a) and body weights (b) of the steers fed concentrate either supplemented (+VIT : ●) or not supplemented (–VIT : ○) with vitamins A, D₃ and E during the fattening period. Bars extended from data points indicate the SEM. Significance of difference between +VIT and –VIT steers : tendency toward significant (* $P < 0.10$).

The changes in plasma retinol concentration are presented in Fig. 2. The plasma retinol concentration in –VIT group steers decreased with time, while the plasma retinol level in the +VIT group did not change during the whole experimental period, and the differences between +VIT and –VIT steers were statistically significant ($P < 0.05$) 6 months after the experiment started. Although a marked de-

cline in plasma retinol level was observed in the –VIT steers, no clinical sign of vitamin A deficiency or other vitamins deficiencies was noticed. Hashimoto *et al.* (Animal Husbandry, 38 : 1138–1142, 1984) reported that vitamin A deficiency symptoms such as staggered gait, convulsion and blindness appeared at the late stage of fattening in dairy steers fed a vitamin A deficient self-formulated concentrate. The

Fig. 2. Changes in plasma retinol concentration of the steers fed concentrate either supplemented (+VIT : ●) or not supplemented (-VIT : ○) with vitamins A, D₃ and E during the fattening period. Bars extended from data points indicate the SEM. Significance of difference between +VIT and -VIT steers : significant (* $P < 0.05$).

duration of vitamin restricted feeding in our study, from 15 to 26 months of age, may not be long enough to manifest the clinical symptoms of vitamin A deficiency. Prolonged fattening without supplemental vitamins would develop vitamin deficiencies.

Slaughtering and carcass measurements are presented in Table 3. Urinary calculi in bladder were observed in 1 out of 3 steers of -VIT group at the slaughter. A slight symptom of sawdust liver was observed in the same animal. Other macroscopic lesional changes were not observed. Slaughter and empty body weights, carcass weight and dressing percentage were not different between the treatment groups ($P > 0.20$). The longissimus area, and thickness of backfat, intermuscular fat and belly measured at the 6-7th cross section was not changed by supplemental vitamins A, D₃ and E. Similar chemical composition of *M. longissimus thoracis* for the +VIT and -VIT groups indicated that restriction of vitamins A, D₃ and E intake during fattening stage from 15

to 26 months old of the steers did not affect deposition of intramuscular fat. This is in contrast to Oka *et al.*²⁴⁾, who reported that the steers without vitamin A administration merited higher marbling grade than the steers receiving intra-muscular vitamin A administration. In their experiments, the steers were slaughtered at 31 to 33 months old. Since marbling is considered to increase with increasing age, relatively slaughter at young age in the present study may be partly responsible for the lack of difference in intramuscular fat content between the treatment groups. It has been reported that retinoic acid interfered at an early stage of preadipocyte differentiation^{7,34)}. Oka *et al.*²⁴⁾ speculated that the restriction of vitamin A intake at an early stage of fattening may improve marbling performance of Wagyu steers through a modulation of intramuscular adipocyte differentiation. In the present experiment, plasma retinol levels were not different between the groups until the steers reached 21 months old. Initiation of vitamins restricted feeding at an earlier stage might contribute to an improvement of marbling performance. If the hyperplasia or differentiation of intramuscular adipocyte was stimulated by vitamin A deprivation, it would be worthwhile to determine the maximal effective stage of manipulation of vitamin A supply with respect to improvement in marbling of fattening cattle. Selected organ weights and abdominal fat depot distribution were not different between +VIT and -VIT steers, although heavier spleen in vitamin A deficient lambs has noticed by Bruns and Webb⁵⁾. There were no significant difference in carcass composition as well as in fat distribution among depots between the treatment groups, although the +VIT steers had non-significant reduced lean (-7%, $P = 0.12$) and increased fat (+13%, $P = 0.17$) proportion compared to the -VIT steers. Since decreased backfat thickness by vitamin A restricted feeding has also been noted in previous experiments (Hodate,

Table 3. Slaughtering measurements, carcass characteristics and carcass composition of the steers fed concentrate either supplemented (+VIT) or not supplemented (–VIT) with vitamins A, D₃ and E¹

Characteristic	Treatment		P-value
	+ VIT	– VIT	
Number of animals	4	3	
Slaughter weight (kg)	651±27	601±19	0.23
Hot carcass weight (kg)	426±19	401±14	0.35
Dressing percentage (%)	65.5±0.8	66.7±0.3	0.28
Empty body weight (kg)	596±24	559±17	0.30
Measurements of 6–7th rib cross section			
Longissimus area (cm ²)	44.0±3.3	43.8±3.1	0.96
Backfat thickness (cm)	3.33±0.33	3.00±0.35	0.52
Intermuscular fat thickness (cm)	6.48±0.61	6.19±0.65	0.77
Belly thickness (cm)	6.90±0.76	6.66±0.31	0.81
Chemical composition of 7th rib portion of M. longissimus thoracis (%)			
Moisture	65.5±1.4	64.2±4.3	0.75
Protein	19.2±0.5	18.8±1.2	0.75
Lipid	13.0±1.6	14.8±5.3	0.74
Ash	0.98±0.01	0.96±0.06	0.69
Organs and abdominal depot fat (% empty body weight)			
Lung	0.44±0.03	0.55±0.09	0.25
Heart	0.39±0.02	0.45±0.04	0.20
Liver	0.88±0.04	0.87±0.02	0.91
Spleen	0.16±0.02	0.15±0.02	0.70
Omental fat	4.34±0.14	4.29±0.11	0.81
Mesenteric fat	5.38±0.64	4.84±0.19	0.52
Kidney fat	1.57±0.24	1.39±0.19	0.61
Carcass composition (%)			
Bone	11.1±0.7	11.2±0.6	0.97
Lean	50.5±1.3	54.3±1.5	0.12
Fat	35.8±1.4	31.8±2.2	0.17
Others	2.6±0.1	2.7±0.1	0.70
Fat distribution in carcass (% carcass fat)			
Subcutaneous fat	41.7±2.5	40.2±3.6	0.73
Intermuscular fat	51.8±3.1	52.9±2.8	0.81
Abdominal surface fat	6.5±1.2	6.9±0.9	0.78

¹ Means±SEM

Proceedings of Japanese Society for Animal Nutrition and Metabolism, 39:157–171, 1995), vitamins restricted feeding may have influenced the carcass composition thorough affecting whole body fat deposition. Antiadipogenic effect of retinol has been demonstrated in murine preadipocyte cell

line^{18,33)} and in pig preadipocyte³⁴⁾ at μM level. On the other hand, Safonova *et al.*²⁹⁾ reported that retinoic acid, an active derivative of vitamin A, stimulated adipocyte formation below $0.01\mu\text{M}$, but inhibited it above $0.1\mu\text{M}$. Vitamin D₃ and its biologically active derivative have been reported to stimulate adipocyte

differentiation at lower concentration below $0.1\mu\text{M}^{3,18}$. Recently, Payne and Watkins²⁷⁾ have reported using growing Coopworth×Dorset wether lambs that the oral administration of retinol and cholecalciferol caused significant reduction in adipocyte diameter and increase in total adipocyte number without any effect on total fat, suggesting an adipose hyperplastic effect of vitamin A and D. Negative correlation between marbling grade and blood vitamin A level has been obtained in several experiments using Japanese beef cattle^{24,36)}. In the present study, however, neither mean plasma retinol level during whole experimental period (15 to 26 months of age) nor for the end of trial (25 and 26 months of age) were related with lipid content in *M. longissimus thoracis*, carcass composition and carcass disectible fat distribution (data not shown). Further study is needed to determine the relationship between vitamins A, D₃ and E nutrition and whole body fatness in beef cattle.

Changes in plasma concentrations of IGF-1, glucose and cholesterol are shown in Fig. 3. There were no treatment effects or consistent age-related changes in plasma concentrations of urea nitrogen (+VIT : 22 ± 0.9 , -VIT : $20\pm0.2\text{mg/dl}$), NEFA (+VIT : 96 ± 7 , -VIT : $78\pm7\mu\text{Eq/l}$) and insulin (+VIT : 132 ± 47 , -VIT : $71\pm2\mu\text{U/ml}$). The +VIT steers had consistently lower ($P=0.08$) plasma cholesterol concentrations than -VIT steers. Hidari *et al.*¹⁵⁾ suggested that the plasma cholesterol level is positively associated with nutrient intake and negatively with activity of *de novo* fat synthesis in fattening steers. In spite of similar feed intake for both groups, lower plasma cholesterol level was observed in the +VIT steers. The mechanism whereby supplemental vitamins A, D₃ and E decrease plasma cholesterol concentration is not known. Both plasma IGF-1 and glucose concentrations were not affected by the treatment, but these were significantly ($P<0.05$) decreased with increasing age. In ruminant, plasma glucose concen-

tration has been reported to increase with increasing amount of concentrate in the

Fig. 3. Changes in plasma concentrations of insulin-like growth factor-1 (IGF-1; a), glucose (b) and cholesterol (c) of the steers fed concentrate either supplemented (+VIT : ●) or not supplemented (-VIT : ○) with vitamins A, D₃ and E during the fattening period. Bars extended from data points indicate the SEM. Significance of difference between +VIT and -VIT steers : tendency toward significant (* $P<0.10$).

ration^{11,22)} and to decrease by fasting^{22,25)}. Blowey and Wood⁴⁾ have reported the usefulness of plasma glucose level as an indicator for monitoring energy intake of dairy herds. Circulating IGF-1 level is known to be growth hormone (GH) dependent, and to increase in response to elevated energy and protein intake^{16,21)}. Kriel *et al.*¹⁹⁾ have shown that the plasma IGF-1 response to GH, which has been abolished by feeding a low-protein diet, was reinstated by intravenous glucose infusion in wether lambs. Gradual decrease in ME intake per unit of MBS was observed in this study. So the gradually decreased plasma glucose level should be due in part to the decrease in energy intake. The decreased plasma IGF-1 might be a reflection of the reduced ME intake and the decreased plasma glucose as the experiment proceeded, although the other regulatory factors such as GH secretion would be concerned.

The responses to NE and glucose challenges are summarized in Table 4. Glucose AUC to NE injection was not changed by vitamin supplementation and stage. Plasma NEFA response to the NE challenge, *i.e. in vivo* lipolytic responsiveness, was not affected by the restricted vitamins intake, but progressively increased ($P < 0.01$) with increasing age. Ozawa *et al.*²⁶⁾ also reported an age-related increase in plasma NEFA response to NE in Japanese Black steers receiving *ad libitum* feeding of high energy concentrate and roughage. Thus, *in vivo* lipolytic responsiveness of Wagyu steers, at least under full fed condition, may represent the degree of progress in fattening. Glucose AUC after glucose infusion was not affected by the restriction of vitamins A, D₃ and E intake or stage. The fractional clearance rate of glucose change during the initial declining phase (0-30 minutes postchallenge) did not differ in the both group of steers,

Table 4. Summary of plasma metabolite and insulin responses to the metabolic challenges¹

Variable	Age (mo)	Treatment		P-values		
		+ VIT	- VIT	Vitamin	Age	Vitamin × Age
Number of animals		4	3			
Nor-epinephrine challenge						
Glucose response area (mg · min · dl ⁻¹)	17	4852 ± 423	3305 ± 97	0.57	0.53	0.37
	21	4874 ± 1020	4898 ± 70			
	25	4709 ± 419	4874 ± 1420			
NEFA response area (μEq · min · l ⁻¹)	17	4008 ± 316	2567 ± 827	0.30	<0.01	0.75
	21	7136 ± 1216	5841 ± 787			
	25	9397 ± 1827	7017 ± 1188			
Glucose challenge						
Glucose response area (mg · min · dl ⁻¹)	17	2010 ± 712	1869 ± 251	0.61	0.39	0.10
	21	2060 ± 654	924 ± 109			
	25	1727 ± 494	1773 ± 640			
Glucose, Fractional rate of change (min ⁻¹)	17	-0.0203 ± 0.0026	-0.0195 ± 0.0016	0.86	0.02	0.78
	21	-0.0218 ± 0.0030	-0.0240 ± 0.0049			
	25	-0.0269 ± 0.0029	-0.0276 ± 0.0029			
Insulin response area (μU · min · ml ⁻¹)	17	26249 ± 9514	6300 ± 180	0.13	0.01	0.64
	21	21829 ± 5242	10628 ± 943			
	25	38826 ± 9180	23360 ± 4786			

¹ Means ± SEM.

although this was rapid ($P < 0.05$) at 25 months of age than at 17 and 21 months of age. Plasma insulin AUC, *i.e.* insulin secretory response was significantly elevated ($P < 0.05$) at 25 months of age. The +VIT steers showed greater insulin AUC than the -VIT steers at all stages, although the difference was not statistically significant ($P = 0.13$). In a vitamin A deprived rat, the impairment of insulin release both *in vivo* and *in vitro* have been reported⁸. Moreover, Higashi *et al.* (Proceedings of Japanese Society for Animal Nutrition and Metabolism, 39 : 129-145, 1995) have reported that both basal level of plasma insulin and glucose induced insulin secretion declined in sheep fed low vitamin E diets. There might be a possibility that vitamins A, D₃ and E restriction decrease insulin secretion of fattening cattle. The elevation of insulin secretory response at the older age in the present study is in agreement with previous reports^{14, 26, 32}.

In summary, Growth performance of Japanese Black fattening steers tended to be impaired by the restriction of vitamins A, D₃ and E intake. Although the tendency for elevated plasma cholesterol for the vitamin unsupplemented steers was observed, other metabolic and hormonal status were not affected by vitamins supplementation. The present results suggest that dietary supplementation of vitamins A, D₃ and E during certain period of fattening does not affect any of carcass characteristics, and vitamin A deprivation during the fattening stage from 15 to 26 months old appears to be ineffective for improvement of marbling performance of beef cattle.

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ビタミン A, D₃, E が黒毛和種去勢牛の肥育成績, 枝肉形質ならびに 脂肪動員能, インスリン分泌能に及ぼす影響

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ビタミン AD₃E 剤の給与制限が肥育牛の飼養成績, 枝肉形質ならびに血中成分濃度および負荷試験(ノルエピネフリン, グルコース)に対する反応に及ぼす影響を調べた。15ヵ月齢の黒毛和種去勢牛をビタミン添加区(4頭), 無添加区(3頭)に群分けし, 濃厚飼料(粗タンパク質 17.3% DM, 代謝エネルギー 3.09 Mcal/kgDM)とイナワラの飽食により 26ヵ月齢まで肥育した。ビタミン添加区にはビタミン A, D₃および E を原物 1kg 当たり 7,500 IU, 1,500 IU および 7.5 IU 添加混合した濃厚飼料を給与し, 無添加区には同一の濃厚飼料にビタミン剤を添加せずに給与した。血漿中レチノール濃度は 21ヵ月齢以降, ビタミン無添加区で有意に低下した。ビタミン AD₃E 剤添加により試験期間中の体重および増体量が大きくなる傾向がみられたが, 飼料摂取量は試験区間で差がなかった。枝肉体組織構成, 枝肉中脂肪の分布, 枝肉歩留, 臓器重量および胸最長筋の粗脂肪含量などの枝肉形質にビタミン添加の有無による有意な差異はみられなかった。ビタミン無添加区で血漿中コレステロール濃度が高い傾向が認められたが, その他の代謝成分およびホルモンの血漿中濃度ならびに脂肪動員能やインスリン分泌能にはビタミン添加の有無による有意な影響は認められなかった。以上の結果から, 15ヵ月齢から 26ヵ月齢の黒毛和種去勢牛に対するビタミン AD₃E 剤給与の制限は, 増体の停滞がみられるものの, 脂肪交雑などの枝肉形質には影響しないことが示唆された。

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