Effects of Vitamin A on Beef Quality, Weight Gain, and Serum Concentrations of Thyroid Hormones, Insulin-like Growth Factor-I, and Insulin in Japanese Black Steers

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(Received September 1, 1997)

Abstract The effects of vitamin A on the beef quality, weight gain, and serum concentrations of thyroid hormones, insulin-like growth factor-I (IGF-I), and insulin in Japanese Black steers were investigated. Eight Japanese Black steers which were 14 months old were divided into two groups : high vitamin A group (H) and low vitamin A group (L). The animals in H were injected with 303 mg of vitamin A intramuscularly every month. All steers were given vitamin A added to the feed (approximately 100 μg/kg feed) at the age of 21-23 and 26-27 months to prevent manifestation of clinical VA deficiency. Although there was no difference in feed intake between H and L, the average daily gain (ADG) in H was greater than that in L. The beef marbling in L was significantly better than that in H. The backfat depth in H was significantly thicker than that in L. The serum IGF-I concentrations in L gradually decreased and after the age of 18 months were significantly lower than those at the beginning of the experiment. The serum triiodothyronine concentrations in L were significantly lower than those in H during some periods. The change in the plasma glucose concentrations after the insulin injections (0.2 U/kg body weight) was similar in H and L. The glucose infusions (0.2 g/kg body weight) caused a marked increase in the plasma insulin concentrations in H and L, and both H and L showed similar areas under curves of plasma insulin levels which were above the basal levels. These results suggested that restricted vitamin A intake led to lower ADG, better beef marbling and lower serum IGF-I and triiodothyronine concentrations.

Anim. Sci. Technol. (Jpn.) 69 (2) : 90-99, 1998

Key words : Vitamin A, Beef quality, Thyroid hormones, Insulin-like growth factor-I, Insulin

It is well known that the restriction of vitamin A causes low average daily gain (ADG) in beef cattle[8,22]. Recently we described that the restriction of vitamin A improved beef marbling significantly[21]. However, the mechanisms how vitamin A influences weight gain and beef marbling are unclear. There are a number of hormones contributing to weight gain and beef quality in beef steers ; insulin is considered to be mainly involved in the fat deposition in ruminants[17,27]. In rats, vitamin A is necessary for the secretion of insulin[7]. Thyroid hormones and insulin-like growth factor-I (IGF-I) are known to be associated with growth or weight gain in ruminants[3,11,15,29]. In addition, triiodothyronine (T 3) and retinoic acid, a derivative of vitamin A, regulate gene transcription by binding to intracellular proteins that are members of the nuclear receptor superfamily of transcription factors[10,12,26].

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In this research, we investigated the effects of low serum vitamin A levels that were not low enough to cause clinical vitamin A deficiency on beef quality and weight gain. We also examined the serum concentrations of thyroid hormones, IGF-I, and insulin to determine the mechanisms how vitamin A acted.

Materials and Methods

Experimental animals and vitamin A supplementation

Eight Tajima strain steers of Japanese Black native to Hyogo prefecture were used (mean weight : 351.1±12.3 kg ; 14 months old). They were divided into two groups : high vitamin A group (H ; n=4) and low vitamin A group (L ; n=4). Each group was penned in a 5×6 meters area and fed the same diet listed in Table 1. The concentrations of β-carotene in the diets were less than 1 mg/kg. The steers in H were administered 303 mg of vitamin A (Chocola A, Eisai, Tokyo) intramuscularly every month. Both groups were given vitamin A added to the feed (approximately 100 μg/kg feed) at the age of 21-23 and 26-27 months to prevent manifestation of clinical vitamin A deficiency. Body weights, withers heights and heart girths of the steers were measured every month. They were slaughtered at the age of 31 months. After chilling for 48 hours, the marbling levels of the carcasses were evaluated between 6th and 7th ribs according to the procedures of the Japan Meat Grading Association (JMGA)[16], and using beef marbling standard numbers (BMS No.) between 1 (the lowest) and 12 (the highest).

Table 1. Composition of the experimental diets

	Months of age		
	14-20	21-25	26-30
Ingredients, % as-fed basis			
Barley	22.6	37.5	44.1
Steam-flaked corn	30.2	25.0	26.5
Wheat bran	22.6	18.8	17.6
Soybean meal	2.3	2.1	0.0
Rice straw dried	22.2	16.7	11.8
Chemical analysis			
Dry matter (DM), %	88.1	87.3	87.0
Crude protein, % of DM	12.3	12.4	11.4
Ether extract, % of DM	3.2	2.8	2.9
ADF[a], % of DM	14.5	12.7	10.5
NDF[b], % of DM	28.3	24.7	23.2
ME, Mcal/kg of DM[c]	2.85	2.97	3.07

[a] Acid detergent fiber.
[b] Neutral detergent fiber.
[c] Calculated from the values in Standard tables of feed composition in Japan (1995).

Analyses of blood constituents

Blood samples were collected via jugular venipuncture 4 to 5 hours after feeding every two months. The serum was obtained by centrifugation and frozen at −40℃ until assayed. The serum levels of glucose and total cholesterol were measured by an auto-analyzer (Model DRI-CHEM 5500, Fuji Photo Film, Tokyo). The serum levels of free fatty acid were determined by using commercial test kits (NEFA-C test, Wako Pure Chemical Industries, Osaka). The serum concentrations of β-carotene[20], vitamin A (retinol) and vitamin E[1,2] were measured by the high-performance liquid chromatography. The radioimmunoassays for the serum thyroxine (T 4), T 3, IGF-I and insulin were performed by using commercial test kits (Amerlex-M T 4, Amerlex -M T 3, Ortho-Clinical Diagnostics, London ; Somatomedin C·II, Chiron, Tokyo ; Insulin Eiken, Eiken Chemical, Tokyo).

Insulin injection and glucose infusion

All steers were administered insulin and glucose at the age of 21 and 29 months. Insulin (bovine insulin, 27.6 U/mg, Sigma, St. Louis) was injected via the jugular veins at the dose of 0.2 U/kg body weight. Blood samples were collected using catheters (CV Catheter Kit ; Nippon Sherwood Medical Industries, Tokyo) placed in the jugular veins every 15 min from 15 min before insulin injections to 195 min after the injections. Glucose solution containing 25% (w/v) glucose was infused via the jugular

veins at a dose of 0.8 ml/kg body weight (0.2 g glucose/kg body weight) over 5 min. Blood samples were collected via the catheters 15 min and 1 min before glucose infusions, and 5, 15, 30, 45, 60, 90 and 120 min after the infusions. Plasma was separated by centrifugation and frozen at −40℃ until assayed.

Statistical analyses

Experimental data are expressed as the mean ±SE. Student's *t*-test or Welch's *t*-test were used to compare the mean values in H and L. The significance of the difference between the two means was tested by Student's *t*-test if the variance was uniform, or by Welch's *t*-test if the variance was not uniform. Differences at $P<0.05$ were considered to be significant.

Results

Body weight, withers height, heart girth and feed intake

The ADG in H was significantly greater than that in L (Table 2). The average withers height changes in the two groups, however, did not show a significant difference. The aver-

Table 2. Intake and performance of the steers in high-vitamin A and low-vitamin A groups

Item	High vitamin A		Low vitamin A	
Number of cattle	4		4	
	mean	s.e.	mean	s.e.
Feed intake, kg/d	7.95		8.02	
Body weight, kg				
Initial	348.8	11.5	353.5	14.8
Final	655.5	25.2	606.8	14.4
ADG, kg/d	0.60*	0.04	0.49	0.02
Withers height, cm				
Initial	121.5	1.0	117.8	1.3
Final	137.8	1.4	132.8	1.9
Change	16.3	2.0	15.5	1.2
Heart girth, cm				
Initial	166.0	0.4	167.5	0.9
Final	216.0	3.1	213.3	3.0
Change	50.0	3.1	45.8	3.4

* $P<0.05$ compared with low vitamin A.

Table 3. Carcass characteristics of the steers in high-vitamin A and low-vitamin A groups

Item	High vitamin A		Low vitamin A	
Number of cattle	4		4	
	mean	s.e.	mean	s.e.
Hot carcass wt, kg	410.7	20.7	391.1	10.8
Backfat depth, cm	3.3*	0.3	2.4	0.2
Lomgissimus muscle area, cm^2	43.5	1.9	46.3	0.5
Beef color, BCS No.[a]	3.8	0.3	3.8	0.3
Marbling score, BMS No.[b]	5.3*	0.6	7.8	0.3

[a] Beef color standard numbers (JMGA 1988).

[b] Beef marbling standard numbers (JMGA 1988).

* $P<0.05$ compared with low vitamin A.

age heart girth change in H tended to be greater than that in L.

The feed intake during the experiment in H and L was similar, 7.95 and 8.02 kg/day.

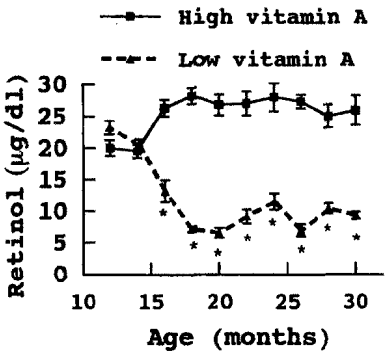

Fig. 1. Serum retinol concentrations of high vitamin A group and low vitamin A group. Vertical bars indicate the standard error. *P< 0.01 compared with high vitamin A group.

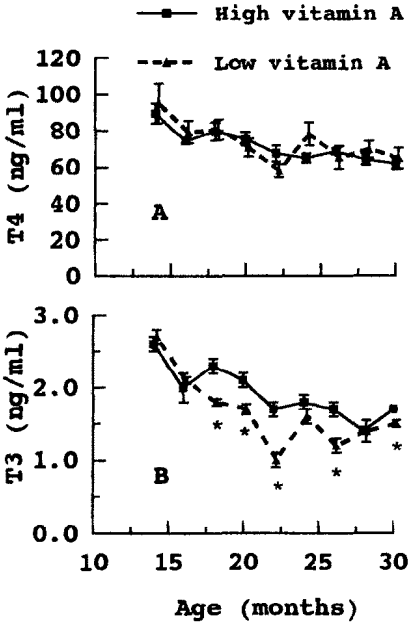

Fig. 2. Serum thyroxine (A) and triiodothyronine (B) concentrations of high vitamin A group and low vitamin A group. Vertical bars indicate the standard error. *P<0.05 compared with high vitamin A group.

All steers showed no clinical signs of vitamin A deficiency such as blindness, edemas of extremities, and neurological symptoms.

Carcass characteristics

The carcass weight in H tended to be heavier than that in L. The mean marbling scores in H and L were 5.3±0.6 and 7.8±0.3, respectively, and were significantly different (Table 3). There was no significant difference in beef color scores between H and L. The longissimus muscle areas in L tended to be greater than those in H. The mean values of back fat depth in H and L were 3.3±0.3 and 2.4±0.2 cm, respectively, and were significantly different.

Blood constituents

The serum retinol concentrations at the start of the experiment in H and L were 19.5±1.0 and 20.4±0.9 μg/dl respectively (Fig. 1). Those in H remained as high as over 25 μg/dl through-

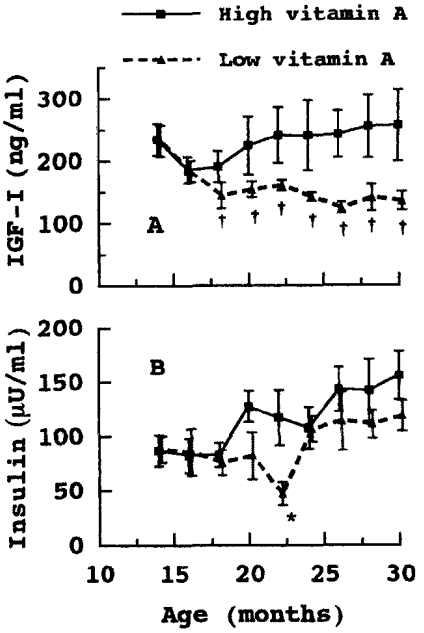

Fig. 3. Serum insulin-like growth factor-I (A) and insulin (B) concentrations of high vitamin A group and low vitamin A group. Vertical bars indicate the standard error. *P<0.05 compared with high vitamin A group. †P<0.05 compared with value at 14 months-old in low vitamin A.

out the experiment, but those in L continued to decrease and reached the minimum of 6.6±0.8 $\mu g/dl$ at the age of 20 months. From the age of 21 months, all steers were given vitamin A (approximately 100μg/kg feed), and thereafter the serum retinol concentrations in L remained at approximately 10$\mu g/dl$. The serum β-carotene concentrations in H and L were similar throughout the experiment, and the serum vitamin E levels in L tended to be higher than those in H at the later stages of the experiment. The serum glucose, total cholesterol and free fatty acid levels showed no significant difference between H and L, and they were within the normal range. Data of the serum concentrations of β-carotene, vitamin E, glucose, total cholesterol and free fatty acid were not shown.

The serum T 4 concentrations in both H and L tended to decrease as the steers grew, and there was no significant difference between H and L (Fig. 2). The serum T 3 levels in both H and L also decreased, but those in L at the age of 18, 20, 22, 26 and 30 months were significantly lower than those in H (Fig. 2).

At the beginning of the experiment, H and L showed similar values of the serum IGF-I concentrations, about 230 ng/ml. Those in L gradually decreased and after the age of 18 months were significantly lower than those in L at the age of 14 months (Fig. 3). Those in H remained constant through the experiment. The serum insulin concentrations in H tended to be higher than those in L (Fig. 3).

Glucose infusion

The plasma glucose and insulin concentrations in H and L markedly increased after glucose infusions, but in 120 min they returned to the pre-infusions levels (Fig. 4). At 5 and 15 min after the glucose infusions, the plasma glucose concentrations in H at the age of 21

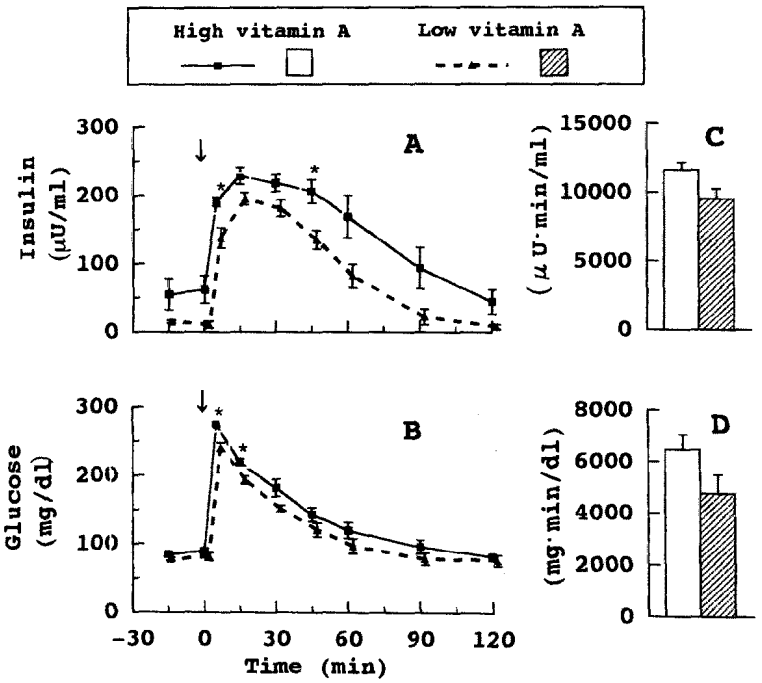

Fig. 4. Plasma concentrations of insulin (A) and glucose (B) and areas under the curves of insulin (C) and glucose (D) before and after infusion (arrows) of glucose (0.2 g/kg of body weight) in high vitamin A group and low vitamin A group at 21 months old. Vertical bars indicate the standard error. * $P<0.05$ compared with low vitamin A group.

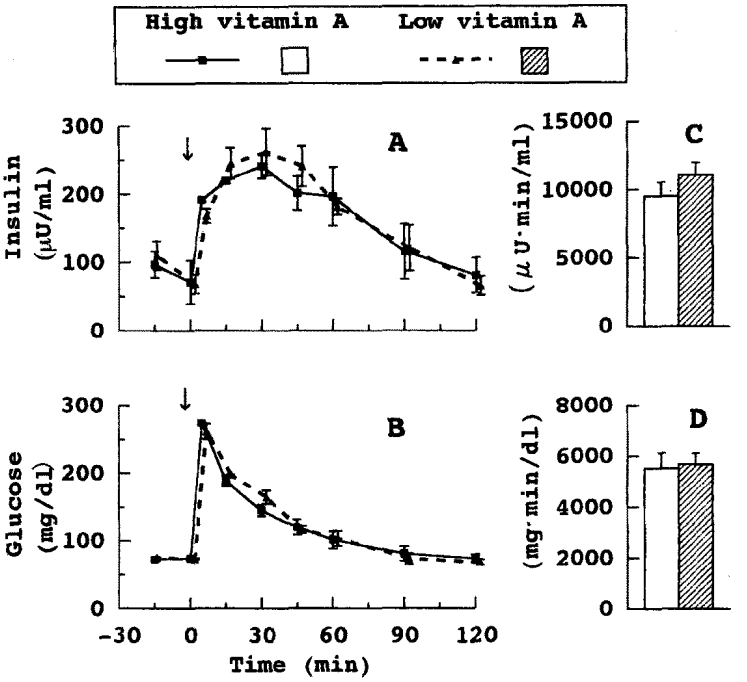

Fig. 5. Plasma concentrations of insulin (A) and glucose (B) and areas under the curves of insulin (C) and glucose (D) before and after infusion (arrows) of glucose (0.2 g/kg of body weight) in high vitamin A group and low vitamin A group at 29 months old. Vertical bars indicate the standard error.

months were significantly higher than those in L. At 5 and 45 min after the infusions, the plasma insulin levels in H were also significantly higher than those in L. The areas under the curves of plasma glucose and insulin levels, which are above basal levels (the means of the values of 15 min and 1 min before glucose infusions) during the period of 5–120 min following glucose infusions, showed no significant difference between H and L. The plasma glucose and insulin levels in both H and L at the age of 29 months varied similarly after glucose infusions (Fig. 5).

Insulin injection

The plasma insulin levels in H and L markedly increased after insulin injections, but there was no significant difference between the two groups (Fig. 6). The increase of plasma insulin levels in H and L at the age of 29 months was greater than that at the age of 21 months. The plasma glucose levels in H and L decreased after insulin injections, and reached the minimum 45–60 min after the injections. Then they gradually increased and returned to the pre-injection levels in 195 min. The plasma glucose concentrations at the age of 21 and 29 months showed no significant difference between H and L.

Discussion

The ADG in H was significantly greater than that in L. Low vitamin A level leads to poor weight gain[8,21,22]. This poor weight gain is considered to be associated with decreased feed intake and feed efficiency (gain/feed)[8]. In this experiment, the reason why the ADG in H was greater than that in L might be the improved feed efficiency, because there was no difference in feed intake.

The serum T3 concentrations in L were

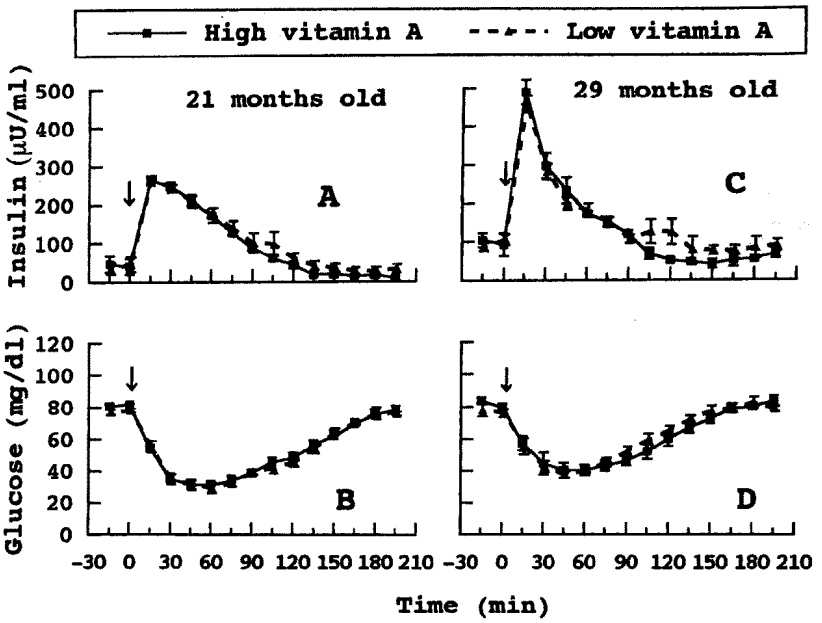

Fig. 6. Plasma concentrations of insulin (A, C) and glucose (B, D) before and after injection (arrows) of bovine insulin (0.2 U/kg of body weight) in high vitamin A group and low vitamin A group at 21 (A, B) and 29 (C, D) months old. Vertical bars indicate the standard error.

significantly lower than those in H during some periods. It is well known that hypothyroidism inhibits the growth of animals, and some investigators reported that the plasma levels of thyroid hormones were positively associated with weight gain in ruminants[15,29]. Therefore it is likely that the decreased T3 in L leads to lower ADG. The serum T4 concentrations did not show a significant difference in the two groups. Morley *et al.*[19] showed that vitamin A enhanced T4 to T3 conversion in hepatic homogenates. Therefore, the effect of vitamin A on T4 to T3 conversion might lead to the higher T3 levels in H.

The serum IGF-I concentrations in H remained constant throughout the experiment, but those in L gradually decreased. Some researchers described that low blood IGF-I levels were caused by low energy intake[6,15,30]. There was no significant difference in feed intake between H and L in the present experiment. Blood free fatty acid levels, which were considered to increase following low energy intake[15,18,23], did not increase in this experiment. Thus it is unlikely that decreased blood IGF-I levels in L were caused by the decrease of energy intake. As circulating IGF-I is produced mainly in the liver[13,28] stimulated by growth hormone[9,18], vitamin A may influence the secretion of growth hormone or liver function. In addition, IGF-I affects feed efficiency[5] and has a positive correlation with ADG[13] or empty body gain[15] in cattle. Therefore, the difference of ADG observed in this experiment might be associated with IGF-I.

The steers in L had more intramuscular fat and less back fat than those in H. A study of Japanese Black bulls showed that back fat did not change significantly from 11 months of age to 80 months of age and that the marbling score increased during 11-30 months of age[14]. This indicated that the deposition of intramuscular fat might be greatly related with

growth of whole body. The deposition of back fat is believed to depend on the nutritional state of the animal[4,25]. Smith *et al.*[25] suggested that different regulatory processes controlled *de novo* fatty acid synthesis in intramuscular and subcutaneous adipose tissue. Thus the mechanisms of the deposition of intramuscular fat and back fat may not be the same. Similarly the effect of vitamin A on the deposition of intramuscular fat and back fat may not be the same, either.

Circulating IGF-I is reported to have a positive correlation with muscular protein accumulation[3,15]. Some reports described the relationship between circulating IGF-I and fat accretion rate[3,15], intramuscular fat content[11] or beef marbling[5,24], but the results were not consistent. Further investigation is necessary to clarify whether IGF-I influences the effect of vitamin A on beef marbling.

Insulin is believed to accelerate fat accumulation. There was a positive correlation between blood insulin concentrations and intramuscular fat in steers[27], and the secretion rates of insulin were greater in obese than lean heifers[17]. In rats, Chertow *et al.*[7] showed that the biphasic insulin release from vitamin A-deficient perifused islets was markedly impaired. In this experiment, although there was no significant difference in insulin secretion after glucose infusions between H and L, the secretion in H whose beef marbling was poor tended to be greater than that in L. This suggests that insulin does not influence the effect of VA on beef marbling.

Glucose is an important lipogenic precursor in the intramuscular adipose depot in steers[26]. McCann *et al.*[17] reported that the reactivities to exogenous insulin in obese and lean heifers were different and that the serum glucose levels after insulin infusions in lean heifers were lower than those in obese ones. In this experiment, however, there was no difference in glucose levels after insulin injections between H and L. This indicates that vitamin A may not influence the tissue reactivity to insulin.

In Japanese Black steers, the restriction of vitamin A intake causes low ADG, better beef marbling and thin backfat depth. This decrease of ADG seems to be affected by T3 and IGF-I. Neither insulin secretion nor tissue sensitivity to insulin could influence the effect of vitamin A on beef marbling.

Acknowledgements

The authors are grateful to Dr. Koichi Hodate, Tohoku National Agricultural Experiment Station, for his helpful advice. We also thank the staff of Hyogo Prefectural Agricultural Institute for their care of the animals and their technical assistance.

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ビタミンAが黒毛和種肥育牛の肉質，増体および血液中ホルモン濃度に及ぼす影響

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ビタミンAの肉質，増体に対する影響を調べ，さらに，ビタミンAの作用機序を検討するために，まず，血液中の甲状腺ホルモン，インスリン様成長因子I（IGF-I）およびインスリン濃度を調べた．14ヵ月齢の黒毛和種去勢牛8頭を用い，高ビタミンA群（4頭）と低ビタミンA群（4頭）に分け，高ビタミンA群にはビタミンA303 mgを毎月筋肉内注射した．21～23および26～27ヵ月齢時には低ビタミンA群のビタミンA欠乏症を防止するためビタミンAを飼料に添加した（約100μg/kg飼料）．血清中ビタミンA濃度は高ビタミンA群は試験期間中25μg/dl以上の高い値で推移したが，低ビタミンA群は20ヵ月齢で6.6±1.6μg/dlまで低下し，それ以降は10μg/dl前後で推移した．飼料摂取量には差が見られなかったが，増体重は高ビタミンA群が重くなった．脂肪交雑は低ビタミンA群が高ビタミンA群よりも有意に高くなった．皮下脂肪厚は高ビタミンA群が有意に厚くなった．血清中IGF-I濃度は低ビタミンA群は徐々に低下し，18ヵ月齢以降は試験開始時と比べ有意に低い値となった．サイロキシンは両群で有意差は認められなかったが，トリヨードサイロニンは低ビタミンA群が有意に低い値を示した時期があった．また，21，29ヵ月齢時にインスリン（0.2 U/kg体重）およびグルコース（0.2 g/kg体重）を負荷し，インスリンおよびグルコース濃度の推移を調べたところ，インスリン負荷による血漿中グルコース濃度の変化には両群の間に差は認められなかった．また，グルコース負荷によりインスリン濃度は両群ともに著しく上昇したが，インスリンの反応下面積は両群に有意な差は認められなかった．以上の結果から，ビタミンAの摂取量を少なくすると脂肪交雑が良くなり，血液中IGF-Iおよびトリヨードサイロニン濃度が低くなることが示唆された．

日畜会報，*69 (2) : 90-99, 1998*

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