

# Serum Vitamin A and Vitamin E in Japanese Black Fattening Cattle in Miyazaki Prefecture as Determined by Automatic Column-Switching High Performance Liquid Chromatography

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**ABSTRACT.** Japanese Black fattening cattle in Miyazaki prefecture, Japan were examined for serum vitamin A (V. A) and vitamin E (V. E) by automatic column-switching high performance liquid chromatography with automated deproteinization. Results indicated that most Japanese Black fattening cattle in Miyazaki prefecture may be provided with V. A supplement and diets including little V. E, moderate  $\beta$ -carotene and V. A during the early fattening stage, and diets including little  $\beta$ -carotene, V. A and V. E during the middle and later fattening stages. Therefore, monitoring serum V. A and V. E in Japanese Black fattening cattle throughout the fattening period seems necessary for farmers in Miyazaki prefecture to avoid economic loss attributable to these deficiencies. — **KEY WORDS:** cattle (Japanese Black), HPLC (automatic column-switching), vitamin A and E.

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Deteriorated meat quality owing to vitamin A (V. A) and vitamin E (V. E) deficiencies is a serious economic problem for farmers with Japanese Black fattening cattle [12]. The low roughage and high concentrate diets provided to Japanese Black fattening cattle in Japan are generally low in  $\beta$ -carotene, V. A and V. E. However, most farmers in Japan provide no V. A supplement to Japanese Black fattening cattle to increase the high beef marbling score (BMS) and V. A status has been reported to correlate with V. E status in Japanese Black fattening cattle [12]. This background may partly account for V. A and V. E deficiencies. Therefore, examining serum V. A and V. E in Japanese Black fattening cattle before slaughter is very important for farmers to avoid economic loss attributable to these deficiencies.

The present study investigated serum V. A and V. E in healthy and V. A deficiency-suspected Japanese Black fattening cattle before slaughter in Miyazaki prefecture, Japan.

Five hundred sixty four healthy cattle were divided into 7 groups based on age. Twelve V. A deficiency-suspected cattle showed clinical signs such as swelling of limbs, hemafecia and blindness. In the present study, a novel high performance liquid chromatography (HPLC), automatic column-switching HPLC (acsHPLC), was used to examine a great number of cases. The acsHPLC method [3] is much simpler for examining a large number of cases than HPLC [1, 4, 5, 8], since deproteinization is automated.

Distilled water and ethanol of HPLC grade, sodium dodecyl sulfate (SDS) of analytical grade and  $\alpha$ -tocopherol were purchased from Wako Pure Chemical Industries, Co., Ltd (Osaka, Japan). Retinol was manufactured by SIGMA (St. Louis, U.S.A.). The components for this method are controller (SC-8020), pump (CCPM-II), degasser (SD-8022), automatic sample injector (AS-8020), automatic switching device (VC-8020), pretreatment column (TSK

precolumn BSA-ODS, 4.6 mm  $\times$  35 mm i.d.), analytical column (TSK-gel ODS-80TS, 4.6 mm  $\times$  150 mm i.d.), guard column (TSK guard column ODS-80TS, 3.2 mm  $\times$  15 mm i.d.) and detector (FS-8020). All these components were manufactured by TOSOH Corp. (Tokyo, Japan). Mobile phases for pretreatment and analysis were 20% and 85% ethanol, respectively. The flow rate for pretreatment and analytical columns was 1.0 ml/min. Emission wave lengths were 460 nm for V. A and 325 nm for V. E; excitation wave lengths were 340 nm for V. A and 298 nm for V. E. The analytical column was maintained at 40°C. Two hundred  $\mu$ l of sample and standards were diluted with 1,800  $\mu$ l of 30% ethanol containing 0.2 M SDS. Two hundred  $\mu$ l of the mixture were automatically injected into the pretreatment column. The mobile phase for pretreatment flowed for 4 min to remove contaminated protein. Small and hydrophobic molecules remaining in the pretreatment column were directed to the analytical column by the mobile phase for analysis. Following detection of V. A, wavelengths were changed automatically to detect V. E. Recovery rates of the V. A and V. E standards from sera were determined by the following equation:

$$\text{recovery rate(\%)} = \frac{(\text{peak area of standard with serum added}) - (\text{peak area of blank serum})}{\text{peak area of standard}} \times 100$$

On HPLC chromatogram, cattle serum showed the same two major separated peaks as the mixture of the V. A and V. E standards 8 and 18 min later, respectively. Areas of the first and second major peaks varied with these concentrations. Mean recovery rates for the V. A and V. E standards from sera at 21–825 IU/dl (n=3) and 100–1125 mg/dl (n=3) were 100% and 105%, respectively. As also reported by Hatam and Kayden [8], internal standards were

not used in this study to attain calibration curves for serum V. A and V. E for the sake of simplicity. Complete linearity of the peak areas plotted against the V. A and V. E standards was demonstrated at 3.3–825 IU/dl and 8.9–1125 mg/dl, respectively. Detection limits for serum V. A and V. E were 3.3 IU/dl and 8.8 µg/dl, respectively, at a signal-to-noise ratio of 3. Coefficients of within-run variation for serum V. A and V. E were 1.0% and 1.9%, respectively (n=6), and for between-run variation, 2.8% for V. A and 5.3% for V. E (n=5). Good correlation of acsHPLC assay with HPLC assay was noted (n=10), the correlation coefficients being 0.83 (p<0.01) for V. A and 0.86 (p<0.01) for V. E. These results revealed the acsHPLC method may be applicable to the simultaneous determination of serum V. A and V. E in cattle. This method is more rapid and efficient, making it possible to reliably handle a greater number of cases.

As the fattening stage proceeded, mean serum V. A in healthy Japanese Black fattening cattle in Miyazaki prefecture decreased. Rosenberger [16] determined that serum V. A below 82 IU/dl and 30 IU/dl were subclinically and clinically deficient, respectively. Thirty two % of cattle 10 to 12 months of age, 29% of cattle 13 to 15 months of age, 55% of cattle 16 to 18 months of age, 67% of cattle 19 to 21 months of age, 98% of cattle 22 to 24 months of age, 94% of cattle 25 to 27 months of age and 98% of cattle 28 to 30 months of age showed subclinically deficient levels. Furthermore, 4% of cattle 13 to 15 months of age, 5% of cattle 16 to 18 months of age, 23% of cattle 19 to 21 months of age, 47% of cattle 22 to 24 months of age, 63% of cattle 25 to 27 months of age and 62% of cattle 28 to 30 months of age showed clinically deficient levels. These results indicated that most farmers in Miyazaki prefecture may provide Japanese Black fattening cattle with V. A supplement and diets including moderate  $\beta$ -carotene and V. A during the early fattening stage, and diets including little  $\beta$ -carotene and V. A during the middle and later fattening stages. Oka [15] recommended the following feeding method to achieve excellent meat quality and daily gain: Japanese Black fattening cattle should be provided with diets including moderate  $\beta$ -carotene and V. A during the early fattening stage, and diets including little  $\beta$ -carotene and V. A during the middle stage. And V. A supplement should be provided to the cattle during the early and later fattening stages. Some Japanese Black fattening cattle in the early fattening stage in Miyazaki prefecture showed low serum V. A levels. This indicated that some farmers in Miyazaki prefecture may provide diets including little  $\beta$ -carotene and V. A to Japanese Black fattening cattle during the early fattening stage. Another possibility is that serum V. A in some Japanese Black fattening cattle in Miyazaki prefecture may be low at the time diets are introduced. Low serum V. A in Japanese Black fattening cattle during the early fattening stage induces increased susceptibility to infection [15]. Serum V. A in Japanese Black fattening cattle in the later fattening stage was lower than recommended for excellent daily gain during the later fattening stage [15].

This indicated that most farmers in Miyazaki prefecture may hesitate to provide V. A supplement to Japanese Black fattening cattle during the later fattening stage to increase the BMS. Another possibility is that increasing serum V. A in Japanese Black fattening cattle showing deficient levels may be difficult and time-consuming. In addition to decreased daily gain, muscular edema is induced by extremely low serum V. A in Japanese Black fattening cattle [14] (Fig. 1).

Normal serum V. E was reported to be 410 to 520 µg/dl by Smith [19]. Sharman [17] determined that serum V. E below 150 µg/dl was deficient. Serum V. E in healthy Japanese Black fattening cattle at all fattening stages in Miyazaki prefecture was below 420 µg/dl. Furthermore, 66% of cattle 10 to 12 months of age, 62% of cattle 13 to 15 months of age, 40% of cattle 16 to 18 months of age, 37% of cattle 19 to 21 months of age, 40% of cattle 22 to 24 months of age, 52% of cattle 25 to 27 months of age and 42% of cattle 28 to 30 months of age showed deficient levels. These results indicated that most Japanese Black fattening cattle may be provided with V. E-low diets. V. E enhances conversion of  $\beta$ -carotene to V. A [22]. This implies that low serum V. E may be associated with low serum V. A. The cattle were not examined for serum creatine phosphokinase (CPK) activity in the present study. However, serum CPK activity in Japanese Black fattening cattle in Miyazaki prefecture was generally high (unpublished data). These suggested subclinical muscular

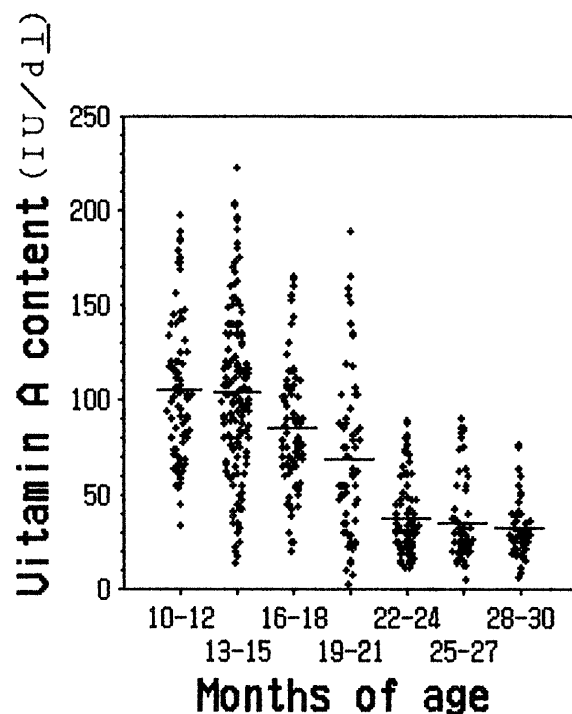


Fig. 1. Serum vitamin A in healthy Japanese Black fattening cattle 10 to 12 (n=80), 13 to 15 (n=146), 16 to 18 (n=80), 19 to 21 (n=64), 22 to 24 (n=81), 25 to 27 (n=61) and 28 to 30 (n=52) months of age in Miyazaki prefecture. Each bar represents mean.

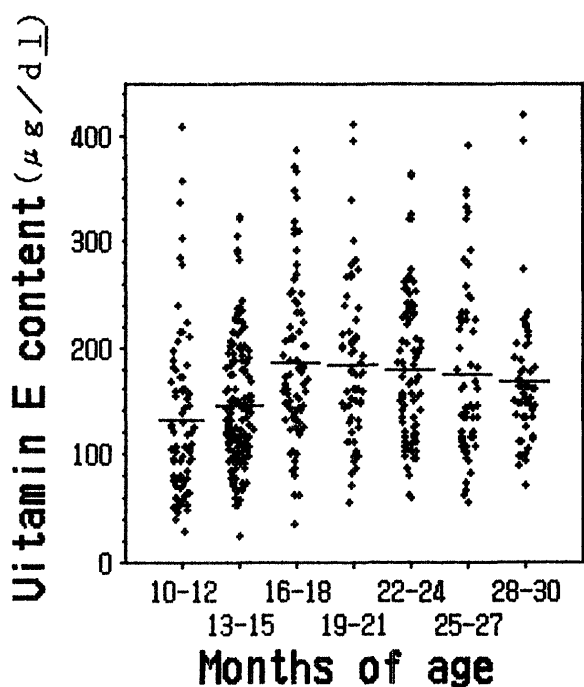


Fig. 2. Serum vitamin E in healthy Japanese Black fattening cattle 10 to 12 (n=80), 13 to 15 (n=146), 16 to 18 (n=80), 19 to 21 (n=64), 22 to 24 (n=81), 25 to 27 (n=61) and 28 to 30 (n=52) months of age in Miyazaki prefecture. Each bar represents mean.

damage attributable to V. E deficiency may occur in Japanese Black fattening cattle in Miyazaki prefecture. (Fig. 2)

Serum V. A was clinically deficient in V. A deficiency-suspected Japanese Black fattening cattle 20 to 32 months of age in Miyazaki prefecture. Serum V. E in these cattle was below 200  $\mu\text{g}/\text{dl}$ . Furthermore, serum V. E was also deficient in 6 cattle (Table 1). Interestingly, serum V. A was clinically deficient in some healthy Japanese Black fattening cattle. Further studies are necessary to clarify the reason.

V. A,  $\beta$ -carotene and V. E are fat-soluble vitamins and bile salts and pancreatic juice are necessary for their absorption. Some  $\beta$ -carotenes are converted to V. A by iron-containing dioxygenase in the intestinal mucosa. V. A and most V. E are transported via the lymph to the liver, where retinol-binding protein (RBP), thyroxine-binding prealbumin (PA) and lipoprotein are synthesized. A small amount of V. E is absorbed through the portal vein. V. A is secreted from the liver to the blood as a complex with RBP and PA. V. E is transported in the blood by lipoprotein [7, 10, 11, 13, 21]. Conversion of  $\beta$ -carotene to V. A is modulated by thyroid [9] and inhibited by nitrate [23]. Zinc deficiency induces decreased RBP [18]. V. E and selenium are complementary antioxidants. Therefore, low selenium induces increased V. E consumption, thereby resulting in low serum V. E. Therefore, iron, zinc and selenium deficiencies, nitrate poisoning, protein-calorie malnutrition and functional disorder of liver, gallbladder, pancreas,

Table 1. Serum vitamin A (V. A) and vitamin E (V. E) in V. A deficiency-suspected Japanese Black fattening cattle

	V. A (IU/dl)	V. E ( $\mu\text{g}/\text{dl}$ )	Age (month)	Clinical sign
Case 1	23	173	28	Swelling of hind limbs
Case 2	13	157	24	Hemafecia
Case 3	28	189	27	Blindness
Case 4	20	160	32	Swelling of hind limbs
Case 5	25	190	24	Swelling of hind limbs
Case 6	18	178	25	Blindness
Case 7	14	66	20	Hemafecia
Case 8	16	137	28	Blindness
Case 9	18	106	27	Blindness
Case 10	8	147	27	Blindness
Case 11	17	136	29	Swelling of hind limbs
Case 12	1	43	27	Swelling of fore and hind limbs

intestine and thyroid may be associated with low serum V. A and V. E. However, healthy Japanese Black fattening cattle examined in the present study showed no signs of these disorders.

V. A and V. E deficiencies induce nyctalopia, urolithiasis, nutritional myopathy [10], nervous disorder [2], nutritional hepatic necrosis [20], increased susceptibility to infection [10, 15], muscular edema, decreased daily gain and deteriorated meat quality. Therefore, monitoring serum V. A and V. E in Japanese Black fattening cattle throughout the fattening period is very important for farmers in Miyazaki prefecture to avoid economic loss attributable to these deficiencies. This may lead to increased routine analysis of serum V. A and V. E, and the acsHPLC method may facilitate reliable handling of a greater number of cases. If serum V. A and V. E in Japanese Black fattening cattle are deficient, providing the cattle with V. A and V. E supplements and diets including sufficient V. A and V. E are recommended.

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