Effects of Serum Vitamin A Level on Tapetum in Japanese Black Cattle Eye

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Abstract

The relationship between serum vitamin A level and tapetum reflection was investigated using ultraviolet imaging. The tapetum was obtained from eyes of slaughtered cattle in which the serum vitamin A level was below 50 IU/dl. The tapetum reflection was examined in the tapetum lucidum, tapetum nigrum, and optic disc. For each part, the tapetum reflection decreased with increasing serum vitamin A level. A high tapetum reflection value might represent the bleached tapetum symptom that occurs because of the low serum vitamin A level.

[Keywords] beef quality, tapetum, ultraviolet camera, vitamin A deficiency

I Introduction

Since serum vitamin A (V.A) inhibits the differentiation of preadipose cells into adipose cells (Pairault et al., 1988; Yano, 1999), serum V.A level affects beef quality depends on the Marbling score (Wheeler et al., 1994; Oka et al., 1998; Adachi et al., 1999; Kruk et al. 2008). Breeders of Japanese black beef are therefore concerned on the control of serum V.A level. To produce high quality meat, serum V.A should be maintained at a low level, approximately 30 IU/dl, in cattle aged 16-24 months. Since V.A deficiency (serum V.A level < 30 IU/dl) can induce serious diseases in cattle, such as nyctalopia, lacrimation, diarrhea, xerophthalmia, convulsive seizures, and other infections (O'Donoghue, 1955; Adachi et al., 1998; National Research Council, 2000), serum V.A level should be monitored carefully to maintain it above 30 IU/dl. The conventional blood assay used to measure serum V.A is time-consuming, expensive, and stressful to cattle, therefore, a non-invasive method is required.

The significant symptom of V.A deficiency is related with an optical lesion. Blindness was associated with the constriction of the optic nerve (Moore, 1941). Rubin (1974)

found that the optic disc became more prominent as the V.A deficiency becomes more severe. Various abnormal changes on the tapetum have also been reported (Kato, 1981, 1983).

It is known that the visual pigment rhodopsin located in the retina on the tapetum contains 11-*cis* isomer of retinal (vitamin A aldehyde) and has absorption peaks at approximately 500 nm and 380 nm (Bownds, 1967). According to Sakamoto and Khorana (1995), maximum absorption shifts from 500 nm to 380 nm following exposure to light. Since the tapetum image can only be captured under light, the image obtained using ultraviolet (UV) light may vary according to the V.A level.

The objective of this study was to investigate the relationship between serum V.A level and the tapetum (retina) reflection using UV imaging.

II Materials

The tapetum reflection was measured on November 19, 2008 and December 11, 2009 at Hyogo Prefecture Meat Inspection Center (Kakogawa, Japan). The number of samples, serum V.A level, and cattle age are shown in Table 1. Because

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the symptoms of V.A deficiency occur at a V.A level below 40 IU/dl (Rubin, 1974) or 50 IU/dl (Kato, 1983), cattle with V.A level below 50 IU/dl were selected for this study.

After measuring serum V.A level using a conventional blood assay, the eyeball was removed randomly from the right or left eye of slaughtered cattle. Then, the tapetum was obtained by cutting the eyeball and removing the glass body.

As shown in Fig. 1, the tapetum includes the tapetum lucidum (a), tapetum nigrum (b), and optic disc (c). Each three area was used to measure the tapetum reflection.

Table 1 Number of samples, serum vitamin A level, and cattle age

Date	Number of samples	serum vitamin A level (IU/dl)	Age (months)
2008/11/19	30	9.70 - 43.0	27 - 33
2009/12/11	19	15.3 - 44.2	24 - 34

Fig. 1 Sample images of the tapetum obtained using an ultraviolet camera. The tapetum was obtained from the eyes of slaughtered cattle. It includes the tapetum lucidum (a), tapetum nigrum (b), and optic disc (c).

III Devices and Methods

1. Experimental Devices

The UV camera used in the present study, which comprises a CCD camera (FC1450, Takex, Japan), UV lens (UV-12.5, Fujinon Co., Inc., Japan), and a ring-shaped UV LED lamp (NS375L-3RLQ, Nitride Semiconductors Co., Ltd., Japan), is shown in Fig. 2. The transmission spectrum of the optics is shown in Fig. 3. The measurement distance was 0.13 m and the UV light intensity, which was measured with a UV radiometer (UVA-365, Custom Co., Japan), was 0.2 W/cm².

The first measurement was conducted under fluorescent lamp (FHF32EXNH, Panasonic Co., Japan) on November 19, 2008. The UV intensity under the fluorescent lamp was approximately 2.0 μ W/cm². Second measurement was performed in darkness on December 11, 2009.

Fig. 2 Ultraviolet camera used in the present study.

Fig. 3 Transmission spectrum of the optics.

2. Methods

The images obtained using the UV camera were processed with an image processing software (WinROOF, Mitani Corporation, Japan) to extract the objective area (tapetum lucidum, tapetum nigrum, and optic disc) from the image and measure the mean grayscale value (Figs. 4 and 5). First, the objective area was detected. Second, the image was binarized with threshold “250” to eliminate the halation by the light. Third, the image was inverted to obtain the mean grayscale value. The relationship between the tapetum reflection and serum V.A level was then investigated.

Fig. 4 Flow chart of image processing to determine the mean grayscale value.

Fig. 5 Tapetum lucidum image processing using WinROOF software. The above tapetum sample was obtained from slaughtered cattle.

IV Results and Discussion

The relationship between the tapetum reflection and serum V.A level in slaughtered cattle with serum V.A below 50 IU/dl was investigated. The measurement environment differed between November 19, 2008, when fluorescent lamp was used, and December 11, 2009, when measurements were performed in darkness. The same relationship was observed between serum V.A level and the tapetum reflection irrespective of the use of fluorescent lamp.

1. Tapetum lucidum

The normal tapetum lucidum in cattle appears blue (Ollivier et al., 2004). The tapetum lucidum reflection decreased with increasing serum V.A level (Fig. 6 (a), $y = -0.51x + 120$ on November 19, 2008, $y = -0.94x + 110$ on December 11, 2009). The coefficient of determination between serum V.A level and the tapetum lucidum reflection was 0.16 ($P < 0.05$) and 0.24 ($P < 0.05$) on the two dates, respectively. The high mean grayscale values at low V.A levels indicate that the tapetum lucidum would be relatively bright. Moore (1939, 1941) found that the tapetum lucidum was bleached in V.A deficient calves. Kato (1981, 1983) also noted that the tapetum lucidum that was observed by direct ophthalmoscopy was fading in dairy cows. Our results appear to be consistent with the previously findings. Further research on the mechanism on the tapetum lucidum bleaching due to the V.A deficiency is required.

2. Tapetum nigrum

As shown in Figure 6 (b), a negative relationship was observed between tapetum nigrum reflection and serum V.A

×: November 19, 2008, △: December 11, 2009

Fig. 6 Effects of serum vitamin A level on reflection of tapetum lucidum (a), tapetum nigrum (b), and optic disc (c) on November 19, 2008 (×) and December 11, 2009 (△). The mean grayscale value was obtained from the tapetum image using an ultraviolet camera.

level ($y = -0.29x + 64$ on November 19, 2008, $y = -0.20x + 32$ on December 11, 2009), and the coefficient of determination was 0.16 ($P < 0.05$) and 0.30 ($P < 0.05$) on the two dates, respectively. V.A deficiency caused a mottled appearance (Moore, 1941; Rubin, 1974), or whitely mottling on the tapetum nigrum (Kato, 1981). Our results showed that the mean grayscale value decreased with increasing serum V.A

level, which seems consistent with the reported whitely mottling on the tapetum nigrum at low serum V.A levels.

3. Optic disc

A negative relationship was observed between the optic disc reflection and serum V.A level (Fig. 6 (c), $y = -1.3x + 120$ on November 19, 2008, $y = -0.76x + 72$ on December 11, 2009), and the coefficient of determination was 0.36 ($P < 0.01$) and 0.38 ($P < 0.01$) on the two dates, respectively. Tojo et al. (1988) pointed out the degeneration of the optic disc because of V.A deficiency. This deficiency can be recognized by the presence of papilledema on the tapetum with the optic disc becoming more prominent (Moore, 1941; Rubin, 1974). O'Donoghue (1955) suggested that papilledema is a cause of blindness due to the edema of the optic papillae, which is the result of venous engorgement caused by increased cerebrospinal fluid pressure. In addition, Rubin (1974) found that the optic disc becomes pinker and paler. Although papilledema was not recognized by tapetum imaging with the UV camera, our results suggest that the optic disc became brighter at lower V.A levels.

As determined by direct ophthalmoscopy, the symptoms of V.A deficiency in young growing calves differ from those in mature cattle (Moore, 1941). Rubin (1974) found that the necrosis of the optic nerve occurred only in growing animals. The effects of V.A deficiency observed on the tapetum may vary according to cattle age in month. Furthermore, the extent of papilledema reflects the duration of V.A depletion, and papilledema congenitally occurred in calves born to dams receiving a ration that was low in V.A (Rubin, 1974). These reports suggest that the effects of V.A deficiency on the tapetum are influenced not only by the recent V.A level but also the ration record for V.A and congenital lesions.

V Conclusions

The relationship between serum V.A level and the tapetum reflection was investigated using UV imaging. The same relationship was observed between serum V.A level and the tapetum reflection irrespective of the use of fluorescent lamp to measure the reflection. Negative relationships were observed between serum V.A level and reflection of tapetum lucidum, tapetum nigrum, and the optic disc. Our results suggest that the tapetum becomes brighter at low V.A levels. Our findings might imply the V.A deficiency observation on the tapetum such as tapetum lucidum bleaching, whitely mottling on the tapetum nigrum, and pinker and paler optic disc. However, the severity of papilledema symptoms detected by UV imaging has not yet been elucidated, and it is essential to investigate the relationship between diagnostic specification of the tapetum and serum V.A level. Since

symptoms of V.A deficiency on the tapetum can occur congenitally and depend on the cattle age and its history of V.A supply from its rations, these factors should be investigated further.

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