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Estimation of Serum Vitamin A Level by Color Change of Pupil in Japanese Black Cattle

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Abstract

Color change of pupil area was investigated in Red, Green, Blue (RGB) and Hue, Saturation, Intensity (HSI) color models from July to November in 2010 and from May to December in 2011 to estimate the serum vitamin A level of Japanese black cattle during their vitamin A controlled stage. A 2CCD camera was used to acquire the eye images. The results showed lower vitamin A level cattle usually accompanied with higher red component value, lower saturation in their eye images. An estimation model was built based on red and green component ratio. The estimation error is about 10 IU/dL. The reasonable result shows the feasibility to estimate the vitamin A level by color change of pupil area in Japanese black cattle.

[Keywords] serum vitamin A, Japanese black cattle, 2CCD camera, tapetum, pupil color, beef quality

I Introduction

To meet the demand in the Japanese market for beef with high marbling scores, Japanese black cattle farmers, generally, control the cattle's vitamin A level. Studies have shown that low levels of vitamin A in cattle can result in a high degree of marbling (Oka *et al*. 1998; Adachi *et al*. 1999; Gorocica-Buenfil *et al*. 2007). Recently, Japanese farmers have begun to active manipulate their cattle's vitamin A level (30-40 IU/dL) during the middle fattening stage: 16 to 24 months (see Fig.1). Thus, it is important for farmers to monitor the vitamin A level during this middle fattening stage.

In addition, maintaining a minimum vitamin A level is critical, since a vitamin A deficiency below this level increases disease susceptibility, such as nyctalopia, xerophthalmia, papilledema, or diarrhea (Moore 1941; O'Donghue 1955; Divers *et al*. 1986; Mason *et al*. 2003). The conventional way of measuring vitamin A levels in cattle is by blood assay. This test is invasive and stressful to the cattle. Besides that, complicated procedures and expensive devices, such as high-performance liquid chromatography (HPLC) system are needed. Thus, an alternative that can estimate vitamin A levels quickly and non-invasively is desirable.

Low vitamin A levels in cattle are known to cause retinal degeneration that can lead to increased tapetal reflection and loss of pigmentation of the nontapetal fundus (Moore 1941; Vandonkersgoed and Clark 1988; Gelatt 2001). As vitamin A deficiency progresses, the disease papilledema can occur, where the optic discs enlarge and become pink and pale. Maggs *et al*. (2008) showed that mottling of the tapetum and pallor of the nontapetum also appear with vitamin A deficiency.

Our laboratory (Takahashi *et al*. 2011) found a negative relationship between vitamin A levels and the reflection of tapetum lucidum, tapetum nigrum and the optic disc using ultraviolet imaging, suggesting hyper-reflectivity in cattle pupils at lower vitamin A levels. To our knowledge, this is the first research to estimate vitamin A levels using pupil color change. The aim of the current study is to (1) measure changes in pupil color, and then (2) estimate the vitamin A levels in Japanese black cattle using the relationship with pupil color during the middle fattening stage.

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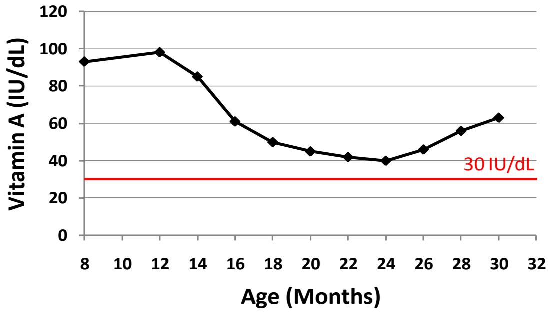

Fig. 1 Ideal serum vitamin A level in cattle during the fattening stage. The red line indicates the minimum desired level (30 IU/dL) of vitamin A in Japanese black cattle. For vitamin A, 1 IU is the biological equivalent of 0.3 μg retinol, or of 0.6 μg beta-carotene. (Provided by Hyogo Prefectural Hokubu Agricultural Institute)

II Materials

This experiment was conducted from July to November in 2010 and from May to December in 2011 with two different groups of cattle at Hyogo Prefectural Hokubu Agricultural Institute, Japan.

In 2010, images of the left eye of 42 Japanese black cattle were taken at 6 different times. The cattle were made up of 22 Yoshihisa-Yoshinaka-Shigeyasu, 15 Nobufu-Teruharu, and 5 locally breed cattle. The measurements were conducted on July 23rd, September 15th, October 14th, October 27th, November 10th and November 24th. The cattle's ages ranged from 15–18 months and at the beginning of the experiment vitamin A levels ranged from 17 to 90 IU/dL (mean level 46$\pm$20 IU/dL).

In 2011, images of the right eye of 42 Japanese black cattle were taken once a month. The cattle were made up of 6 breeds (Table 2) and divided into two groups by date of arrival. Group A consisted of 24 cattle: "Hiroiwadoi", "Yoshigakidoi" and "Hiromi-Yoshigaki" breeds. They arrived at the feedlot in December 2010, their ages ranged from 14–16 months and at the beginning of the experiment their vitamin A levels ranged from 35 to 80 IU/dL (mean level 59$\pm$11 IU/dL). Group B consisted of 18 cattle: "Miyagikujyo", "Kikuyu-Hiromasa -Miyagikujyo" and "Kikuyu- Hiromasa" breeds. They arrived at the feedlot in February 2011. Their ages ranged from 12–14 months, and at the beginning of the experiment their vitamin A levels ranged from 35 to 80 IU/dL. From the beginning of April 2011, the cattle were subjected to a vitamin A free-diet. All the cattle used in this study were clinically healthy during the experimental period.

III Devices and Methods

1. Experimental devices

A 2CCD multi-spectral camera AD-080CL (JAI) was used to acquire both color and Near Infrared (NIR) images. The camera's spectral sensitivity is shown in Fig. 2. Light entering the lens was separated into two light beams by a beam splitter; one was focused on a color image sensor and the other was focused on a monochrome image sensor. The color image sensor was a single-chip image sensor with Bayer filters. The monochrome image sensor was equipped with a filter to capture a NIR image in the wavelength range 750–900nm. The image was 1024 pixels in width and 768 pixels in height. The lens was a TF15D-8 (Focus Length 15 mm, F 2.2, FUJINON) in 2010 and NT63-240 (Focus Length 12 mm, F 1.8, EDMUND) in 2011. The camera was combined with two ring-shaped LED lights; a MDRL-CW50 (MORITEX) white LED light and a MDRL-CIR31 (MORITEX) NIR LED light which has a central wavelength of 850 nm. Two polarizing (PL) filters were installed to reduce the specular reflection from the surface of the eye. One of them was installed in front of the LED, the other in front of the camera, perpendicular to the former PL filter. A plastic tube was installed in front of the LED lights to exclude ambient light. The sketch of the device is shown in Fig. 3. Figure 4 shows the appearance of the device.

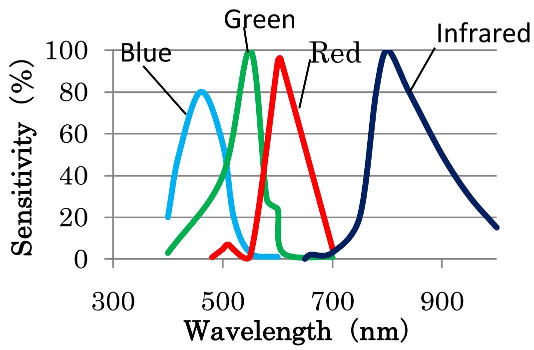

Fig. 2 Total spectral sensitivity of camera including prism and sensor.

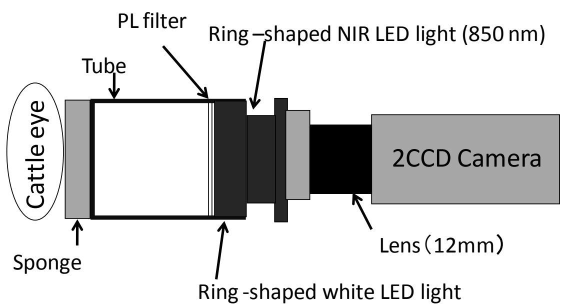

Fig. 3 Sketch of experimental device.

Fig. 4 Appearance of experimental device.

Fig. 5 Color and NIR images of cattle's right eye.

2. Method

Color calibration was performed prior to each experiment to ensure high quality images and minimize operation error. System setting and color calibration was carried out according to the procedure shown in Table 1.

Table 1 Machine vision system setting procedure.

NO.	Setting
1	Set the brightness at the end of tube to 1700 lx
2	Set the focus to 8 mm from the end of tube
3	Set F value to 2.2 in 2010 and 1.8 in 2011
4	Set shutter speed to 1/30 s
5	Set master gain to 336
6	Check white balance and calibrate camera by adjusting red and blue gain
7	Take images of Color Checker (color calibration board)

After calibration, the cattle's eyes were covered for two minutes to adapt to a dark environment. This black cloth cover was kept on until the eye images were captured. Then, we adjusted the aim of the camera to the cattle's eye using NIR-LEDs lighting. After a focused image of the pupil was obtained on the NIR video monitor, the white LEDs were turned on and the eye images were captured simultaneously. Figure 5 shows sample images. Blood assays were conducted one to two days before image acquisition. Vitamin A levels in the blood were determined by HPLC.

The images were processed by software developed in Microsoft Visual Studio 2008 to obtain the color information. The yellow color in figure 6 shows the region of interest (pupil area). The region of interest was determined by the hue value of the color image and the brightness of the NIR image. As shown in figure 5, very little NIR light was reflected from the tapetum, hence the NIR images were not used in any subsequent analysis.

Fig. 6 Original image (Left), Region of Interest shown in yellow color (Right).

A comparison of the color change in RGB color model and HSI color model based on Gonzalez *et al*. (1992) was conducted. Red (r), green (g), and blue (b) component ratios were calculated by the formulas below:

$$r = R/(R+G+B) \quad (1)$$

$$g = G/\,(R+G+B) \quad (2)$$

$$b = B/\,(R+G+B) \quad (3)$$

where R, G and B represent red, green and blue, respectively. They have been normalized and are in the range [0, 1]. The HSI color space is claimed to be the closest approximation to a human interpretation of colors. There are many different models for HSI color space. Here, we use the triangular pyramid model. The calculation method is shown below.

$$H = \begin{cases} \theta & if\ B \le G \\ 360 - \theta & if\ B > G \end{cases} \ with$$

$$\theta = \cos^{-1}\left\{ \frac{\frac{1}{2}[(R-G)+(R-B)]}{[(R-G)^2+(R-B)(G-B)]^{\frac{1}{2}}} \right\} \quad (4)$$

$$S = 1 - \frac{3}{(R+G+B)}[\min{(R,G,B)}] \quad (5)$$

$$I = \frac{1}{3}[R+G+B] \quad (6)$$

To make "Saturation" and "Intensity" easy to interpret, they are multiplied by 100, and 255, respectively. So they are in the range of [0,100] and [0,255], respectively.

IV Results

1. Relationship between individual color factors and serum vitamin A in 2010

In 2010, the vitamin A level in all the cattle declined and then stayed at a low level around 20–30 IU/dL. By the end of the experiment, the cattle's vitamin A levels ranged from 7 to 35 IU/dL (mean level 19 ±7 IU/dL).

Figure 7 shows changes in vitamin A (mean ± std) and *r* (mean ± std) over time. This indicates that when vitamin A drops, *r* would increase.

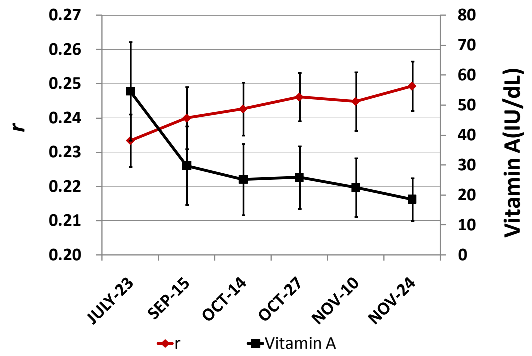

Fig. 7 Changes in vitamin A and red component ratio from July to November 2010. Error bars indicate the standard error of mean (n=42).

In 2010, only average vitamin A levels on July 23rd (mean level 46±20 IU/dL) were significantly above 30 IU/dL. In subsequent months, levels were around or below 30 IU/dL. A comparison between data on July 23rd and November 24th was conducted. This represents the highest and lowest levels of vitamin A, respectively. The results are shown in Fig. 8. The *r* on July 23rd were significant lower than that on November 24th ($p<0.001$). This indicates there is a negative correlation between *r* and vitamin A. Saturation was also investigated in the same way. The results show that vitamin A deficient in cattle is usually accompanied by a lower saturation value, as shown in Fig. 9. The significant color change in the pupil area indicates that changes in the tapetum and retina induced by low vitamin A levels can be detected by measuring the reflected light from the back of the pupil.

To confirm these findings, a similar experiment was conducted again in 2011 with another group of cattle. This time the experimental period was extended from the 5 months to 8 months in order to cover a wider range of vitamin A levels. In addition, a new lens with a smaller F value (1.8) was used in 2011 to acquire brighter eye images, and thus make it easier to detect color changes.

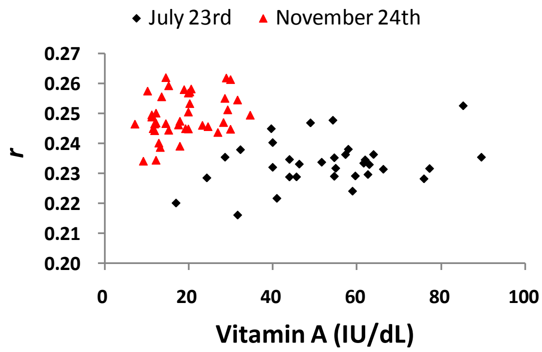

Fig. 8 Relationship between red component ratio and vitamin A level in July 23rd and November 24th 2010.

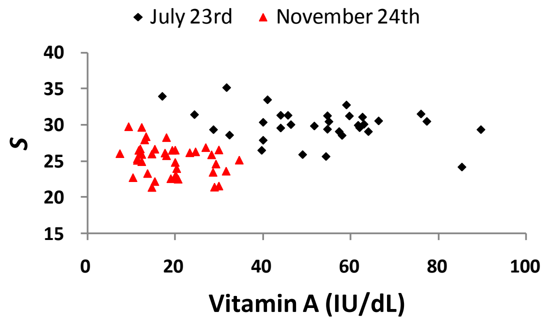

Fig. 9 Relationship between saturation and vitamin A level in July 23rd and November 24th 2010.

2. Relationship between individual color factors and serum vitamin A in 2011

Vitamin A levels in all the cattle gradually declined during the experimental period in 2011. By the end of the experiment, the experiment cattle's vitamin A levels ranged from 11 to 41 IU/dL (mean level 28±7 IU/dL).

The relationship between vitamin A level and *r*, *g*, *b*, hue, saturation and intensity were investigated. Figure 10 shows the correlation between *r* and vitamin A level from May (12–16 months old) to December (19–23 months old) 2011. The correlation is weak. Similar to the results in 2010, lower vitamin A levels tend to be associated with a higher red component. As the vitamin A level dropped, the red color component value increased. The changes in vitamin A level (mean ± std) and *r* (mean ± std) over time are shown in Fig. 11. This data also show that as vitamin A levels decline, *r* rises.

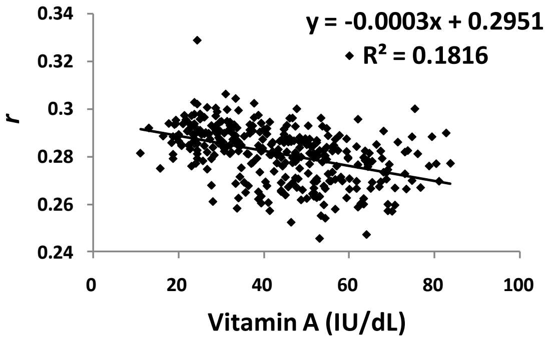

Fig. 10 Relationship between red component ratio and vitamin A level.

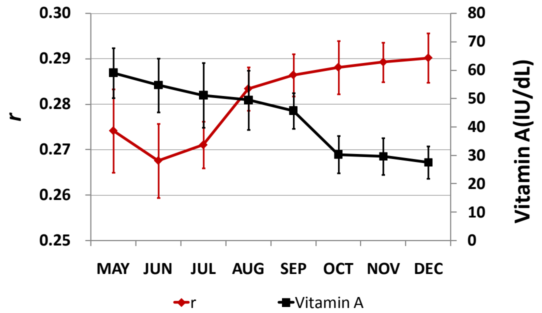

Fig. 11 Changes in vitamin A and red component ratio from May to December 2011. Error bars indicate the standard error of mean.

According to past veterinary experience, at the beginning of the fattening stage serum vitamin A levels of cattle will be around 80 IU/dl. After 3 months of a vitamin A-free diet, vitamin A levels will be around the critical 30 IU/dl value. The symptoms of vitamin A deficiency may not show for another 3 months. Based on this information, we choose the data in May, August and December; corresponding to the beginning, middle and end of middle fattening stage, for correlation analysis. The correlation between color factors (*H*, *S*, *I*, *r*, *g*, *b*) and vitamin A level in the selected three months were investigated. Table 2 shows the correlation coefficients between color factors and vitamin A levels. In Group A, the correlation coefficients of *r*, *S*, *H* were higher than 0.6, indicating a strong correlation. Generally, cattle with lower vitamin A levels had higher *r*, *H*, *I* and lower *b*, *S*, as Table 2 shows. This result for *r* and *S* was consistent with the result obtained in 2010. This higher correlation was obtained using the HSI color model.

Group B showed a weak correlation between color factors and vitamin A levels, where all the correlation coefficients were lower than 0.5. Compared to Group A, cattle in Group B had significantly higher vitamin A levels, especially in October, November and December (p<0.01), as shown in Table 3. It is possible that Group B had more vitamin A storage in their body, so the time taken to deplete vitamin A stores took longer. Which in turn means the detection of color changes will take longer.

Table 2 Correlation coefficients between color factors and vitamin A levels.

	Breeds	Number of cattle	*r*	*g*	*b*	*H*	*S*	*I*
	Hiroiwadoi	8	0.52^-	0.76^+	0.23^+	0.86^-	0.56^+	0.68^-
A	Yoshigakidoi	4	0.75^-	0.50^+	0.65^+	0.82^-	0.75^+	0.58^-
	Hiromi-Yoshigaki	12	0.68^-	0.29^-	0.56^+	0.70^-	0.68^+	0.57^-
	Group A	24	0.60^-	0.51^+	0.42^+	0.77^-	0.62^+	0.55^-
	Miyagikujyo	4	0.27^-	0.09^-	0.29^+	0.05^-	0.28^+	0.65^-
B	Kikuyu-Hiromasa -Miyagikujyo	5	0.39^-	0.08^+	0.30^+	0.21^-	0.39^+	0.24^-
	Kikuyu-Hiromasa	9	0.17^-	0.44^+	0.01^+	0.45^-	0.17^+	0.65^-
	Group B	18	0.21^-	0.28^+	0.09^+	0.33^-	0.21^+	0.55^-
	Overall	42	0.42^-	0.38^+	0.27^+	0.55^-	0.42^+	0.55^-

Note: "+" indicates positive correlation; "-" indicates negative correlation.

Table 3 Serum vitamin A levels from August to December.

	Breeds	NO.	Aug	Sep	Oct	Nov	Dec
	Hiroiwadoi	8	44±5	45±6	25±3	26±3	26±5
A	Yoshigakidoi	4	44±4	45±6	28±3	21±2	25±4
	Hiromi-Yoshigaki	12	39±8	39±5	26±5	24±4	24±4
	Group A	24	42±9	42±7	26±5	24±4	25±6
	Miyagikujyo	4	57±6	47±5	39±5	37±4	31±3
B	Kikuyu-Hiromasa -Miyagikujyo	5	58±5	54±8	39±6	39±7	36±4
	Kikuyu-Hiromasa	9	62±10	50±7	33±7	35±8	28±6
	Group B	18	60±10	51±9	36±9	37±8	31±7
	Overall	42	49±13	46±9	30±9	30±9	28±7

Unit: IU/dL.

3. Result of Multiple Linear Regression (MLR) Analysis

To estimate vitamin A levels, a MLR model of vitamin A levels and color factors was developed in Excel 2007. To build the calibration models, the data at three sampled dates (May, August and December) for sixteen Group A cattle were used, and the data at three sampled dates (May, August and December) for 8 Group A cattle were used for validation. The results are shown in Table 4.

Table 4 Multiple Linear Regression Models.

Color Factors	MLR Model	R^2
r,g	$y = -915x_r + 2372x_g - 465$	0.67*

Note: * $p < 0.05$, where x_r, x_g, is the value of *r*, *g* respectively; *y* is the estimated serum vitamin A level.

The "*r, g*" model was used to estimate the vitamin A values of the other 8 cattle. The performance of the "*r, g*" model was evaluated in terms of root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP), and the coefficient of determination (R^2). The results are shown in Table 5. Scatter plots of the "*r, g*" models for calibration and validation are shown in Fig. 12. The data are evenly distributed around the regression line with a 45° slope, which indicates estimated and measured values are very close to each other.

Table 5 Result of "*r, g*" estimation model.

Model	RMSEC (IU/dL)	R^2(Calibration)	RMSEP (IU/dL)	R^2(Validation)
r, g	9.3	0.67	10.3	0.62

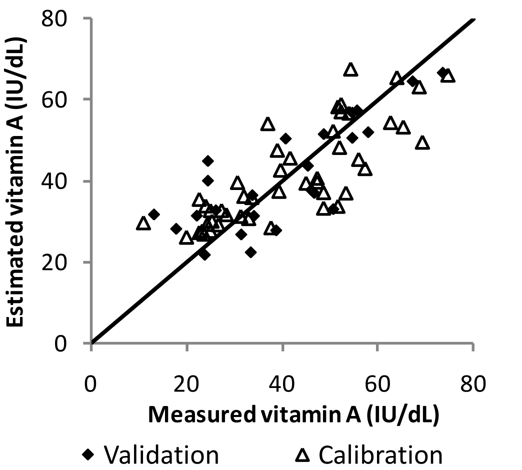

Fig. 12 Measured and estimated vitamin A by "*r, g*" model.

V Discussion

The result shows that cattle with a lower vitamin A level usually have a lower pupil saturation and lower blue component value. The decreased saturation, a measure of the degree to which pure color is diluted by white light (Gonzalez *et al*. 1992), may indicate more white LED light is reflected by the tapetum when there is vitamin A deficiency. As the tapetum is blue in color, a low blue component value for cattle with a low vitamin A level may indicate the mottled appearance of the non-tapetal area because of retina degeneration (Maggs *et al*. 2008). However, this result is based on the subjective notion that the main factor impacting color changes in the eyes of Japanese black cattle is associated with vitamin A levels. Further research, with a vitamin A supplement group, is need to confirm pupil color changes are caused by vitamin A levels, and not other factors, such as age.

The RMSEP (about 10 IU/dL) may be affected by the individual differences between cattle. To decrease the effect of individual differences on the estimation error, it may be effective to conduct a blood assay of vitamin A levels at the beginning of vitamin A control stage. Thus, the estimation error could be reduced based by taking into account color changes and initial vitamin A levels. According to the Japan Livestock Technology Association, three cattle blood assays are needed to identify vitamin A deficiency during the middle fattening stage. With the suggested sampling regime, blood assays could be reduced to 1. Issi reported that the loss of pupillary light reflexes is also a clinical sign of vitamin A deficiency (Issi and Gül 2010). To make the estimation procedure more robust and accurate, other factors such as pupillary light reflex and surface reflection, which may be affected by low vitamin A levels, should also be investigated in the future.

VI Conclusions

A negative correlation between the red component ratio of cattle pupils and vitamin A levels, a positive correlation between the saturation value of cattle pupils and vitamin A levels were found in 2010, and confirmed in 2011. For 24 cattle, with a wider range of vitamin A levels than that in 2010, a multiple linear regression analysis was conducted, and a model $y = -915x_r + 2372x_b - 465$ based on the red and green component ratio was selected. The coefficient of determination for calibration and validation was 0.67 and 0.62, respectively. RMSEC was 9.3 (IU/dL) and RMSEP was 10.3 (IU/dL). This model may not be applicable to other cattle breeds, but the results show the feasibility of estimating vitamin A levels using the color signatures of Japanese black cattle during the middle fattening stage, when their vitamin A levels are depleted. This information derived from pupil color changes will be a valuable aid to farm management.

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