

Preliminary system of rapid analysis of blood retinol level in cattle

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Abstract

Control of blood retinol levels in cattle during fattening is important in the production of marbled beef. However, it is difficult to easily measure the blood retinol concentration in the field. In this study, we attempted to develop an analysis method that does not require blood cell separation and uses a compact fluorescence analyzer that can be carried around as a preliminary system for measuring blood retinol concentration in the field. This system was used to monitor blood retinol levels in 12 fattening cattle (14 to 27 months old) and demonstrated a strong correlation ($r = 0.78$) with the results obtained by the standard high-performance liquid chromatography (HPLC) method. Stronger correlations ($r = 0.87$) were obtained until the cattle were 24 months of age. These results suggest that higher correlations can be expected to be obtained by improving the robustness of the extraction system. Refinements for practical use need to be considered, but whole blood extraction and the vitamin A analyzer that was developed show potential to be used for on-farm monitoring of retinol levels.

KEYWORDS

blood retinol level, cattle, marbling beef, point-of-care testing, smart agriculture

1 | INTRODUCTION

In the production of beef cattle, marbling greatly affects the selling price of beef. Wagyu beef is classified into a total of 15 categories in Japan, with yield grades from A to C, and meat quality grades from 1 to 5 (Japan Meat Grading Association, 2014). Marbling is a major determinant of meat quality grade (beef marbling standard [BMS]). The other three categories are meat color, meat firmness and texture, and fat color and quality. The grading of Wagyu beef is described in detail in the carcass trading standards of the Japan Meat Grading Association and in the report by Motoyama et al. (Japan Meat Grading Association, 2014; Motoyama et al., 2016). For example, the

trading price of Wagyu steers in the Tokyo wholesale market in December 2020 was 2906, 2650, and 2387 JPY/kg for A-5, A-4, and A-3 grades, respectively (Japan Meat Market Wholesaler's Association, 2020). Therefore, many Wagyu farmers aim to maximize intramuscular fat (marbling) in the finishing of their beef cattle. By adjusting the feed, vitamin A (retinol) levels in the blood can be controlled at low levels close to the physiological limits of the cattle, facilitating marbling (Adachi et al., 1999; Arnett et al., 2008; Gorocica-Buenfil, Fluharty, Bohn, et al., 2007; Gorocica-Buenfil, Fluharty, Reynolds, & Loerch, 2007; Oka et al., 1998; Pickworth et al., 2012). Retinoic acid, which is a derivative of vitamin A, has a function to regulate the adipogenic differentiation of fibroblasts

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(Castro-Muñozledo et al., 1987; Kuri-Harcuch, 1982). Vitamin A restriction also restricts retinoic acid, resulting in promotion of adipogenic differentiation of fibroblasts and higher fat hybridization. However, at present, farmers cannot easily measure blood retinol levels, and feed their raw materials in the rations according to empirical feeding methods, without any feedback from blood retinol levels. As a result, stringent control of retinol levels in the blood cannot be achieved, representing a possible reduction in the price of the dressed carcass due to low marbling. Controlling retinol at physiological limits also indicates possible deficiency, leading to immunodeficiency and death (Adachi et al., 1997; Yano et al., 2009), with obvious negative implications for farm income and management.

Usually, blood retinol levels are measured by high-performance liquid chromatography (HPLC) using serum or plasma (Arnaud et al., 1991; LeBlanc et al., 2004; Yang et al., 1992). Using this method, blood retinol levels are obtained by collecting the supernatant from a centrifuged blood sample, using a vacuum collection tube. They are also subjected to multiple pretreatments before being measured by HPLC. Therefore, without specialized skills and knowledge, blood collection and pretreatment such as blood cell separation cannot be performed, and the analysis equipment is large and very expensive, making it impractical for farmers to perform this. One option is to ask a professional organization to perform the analysis. However, there is a time lag, ranging from days to weeks (depending on the organization), before the results of the analysis are returned to the farmers, making it difficult for all farmers to get the results in a short time as required. As an alternative to HPLC, a simple method was developed that combines solvent extraction from plasma and serum with fluorescence and absorbance measurements (Futterman et al., 1975; Suzuki & Katoh, 1990). Miyamoto et al. improved these methods and reported that fluorescence measurement of serum diluted 20-fold with 1-M sodium chloride at an excitation light of 325 nm and detection wavelength of 463 nm showed a high correlation ($r = 0.93$) with the results measured by HPLC (Miyamoto et al., 2003). However, these methods also require blood cell separation and are not suitable for analysis in the field. Therefore, in this study, we attempted to develop an analysis method that does not require blood cell separation, using a compact fluorescence analyzer that can be carried around as a preliminary system for measuring blood retinol concentration in the field, and the practical feasibility of the system was verified.

2 | MATERIALS AND METHODS

2.1 | Animal care

The management of the cattle and all the procedures in the present study were performed in accordance with the Animal Experimental Guidelines of Nagasaki Agriculture and Forestry Technical Development Center (reference number: 30nougichikudai5go) and the National Institute of Advanced Industrial Science and Technology (AIST) (reference number: A2018-0258, A2019-0258).

In this study, all three experiments were conducted using Japanese Black beef cattle.

2.2 | Reagents and apparatus

n-Hexane (085-00416; FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) was used for direct extraction in the experiment in AIST. *n*-Hexane (080-03423; FUJIFILM Wako Pure Chemical Corporation) was used for direct extraction and in HPLC measurement, which were conducted in Nagasaki Agriculture and Forestry Technical Development Center. A sodium heparin-coated vacuum extraction tube (VP-H100K; Terumo Corporation, Tokyo, Japan) was used for drawing blood from cattle. Distilled water obtained from a water distillation unit (SA-2100A; TOKYO RIKAKIKAI CO, LTD, Tokyo, Japan) and ethanol (052-03343; FUJIFILM Wako Pure Chemical Corporation) were used for pretreatment for HPLC measurement. *Bitarongu* (Kyoritsu Seiyaku Corporation, Tokyo, Japan) was used for vitamin preparation to be administered to cattle. A vortex mixer (120209; Heathrow Scientific, IL, USA) and a 1.5-ml microtube (02-681-320; Thermo Fisher Scientific K.K., Tokyo, Japan) were used for mixing whole blood and *n*-hexane. A quartz cell (F15-UV-10; GL Sciences Inc., Tokyo, Japan) and a quartz test tube (QTST-10075; AS ONE Corporation, Osaka, Japan) were used for fluorescent measurement. A centrifuge (5500; KUBOTA Corporation, Tokyo, Japan) was used for centrifugation. A fluorescence spectrophotometer (F2700; Hitachi High-Tech Science Corporation, Tokyo, Japan) was used for principle verification of the direct extraction fluorescence measurement method. HPLC (LC-2000 Pluse series; JASCO Corporation, Tokyo, Japan) was used as a reference method to measure blood retinol level. C18 column (Shodex; Showa Denko K.K., Tokyo, Japan) was used for HPLC measurement. A universal shaker (SHK-U4; IWAKI CO., LTD., Tokyo, Japan) and a centrifugal evaporator (RD400; Yamato Scientific Co., Ltd., Tokyo, Japan) were used for HPLC measurement pretreatment.

2.3 | Direct extraction from whole blood and fluorescence measurement

Plasma or serum is usually used for blood analysis. This is because hemolysis occurs when blood is mixed with extraction or analysis reagents, and the unwanted substances eluted from the red blood cells may affect the analysis. The standard HPLC measurements for retinol require mixing the plasma or serum with an alcohol (or equivalent) that can be mixed with an extraction reagent such as *n*-hexane. This is because plasma or serum is a polar solvent and do not mix well with nonpolar extraction reagents (Arnaud et al., 1991; Yang et al., 1992). Because the nonpolar solvent will then mix well with the aqueous phase, it is necessary to perform phase separation by centrifugation or other means to obtain the organic phase after mixing. For this reason, blood analysis requires centrifugation. Processes such as centrifugation and the collection of supernatants

using pipettes should be eliminated if the process is intended to be implemented in the field. Because *n*-hexane is a nonpolar solvent, it hardly mixes with blood, which is a polar solvent. Therefore, the risk of hemolysis can be avoided even if *n*-hexane and whole blood were mixed directly. Retinol would be extracted at the interface between hexane and blood.

Ten milliliters of whole blood was collected from the jugular vein of cattle using a sodium heparin-coated vacuum blood collection tube. Whole blood (100 μ l) and *n*-hexane (1 ml) were added to a 1.5-ml microtube. The mixing ratio of whole blood and *n*-hexane was based on the mixing ratio of serum and distilled water in previous work (Watanabe et al., 2008), and the mixing ratio of plasma and *n*-hexane in the standard HPLC measurement procedure (National Agriculture and Food Research Organization, 2018) Due to complete separation, samples were stirred with a vortex mixer at 2000 rpm for 10 min. Whole blood and *n*-hexane separated as soon as the agitation stopped, and *n*-hexane was collected by pipette. The *n*-hexane was transferred to a quartz cell for measurement in a fluorescence spectrophotometer. The quartz cell and quartz test tube were specific to the fluorescence spectrophotometer and vitamin A analyzer, respectively. The mixing time was determined to be 10 min because 10 min showed the highest fluorescence intensity when compared with mixing times of 1, 5, and 10 min in a simple test beforehand (Figure S1). A mixing time longer than 10 min was not considered because it is not in appropriate in field use.

2.4 | HPLC measurement

HPLC measurement was used as a reference method. The HPLC procedure was based on the manual published by National Agriculture and Food Research Organization (NARO) with some modifications (National Agriculture and Food Research Organization, 2018). The remaining whole blood (i.e., that which was not used for direct extraction) was subjected to centrifugation at 3000 rpm (1690 g) for 10 min at room temperature, and the supernatant was collected to obtain the plasma. Plasma was saponified by mixing 0.5 ml of plasma with 0.5 ml of distilled water and 1 ml of ethanol. Retinol was extracted by mixing it with 5 ml of *n*-hexane for 10 min. This was then centrifuged at 2000 rpm (751 g) for 5 min at room temperature, and 4 ml of supernatant was collected. The cell containing this supernatant was heated to 40°C in a centrifugal evaporator and distilled. This was dissolved in 100 μ l of methanol and analyzed by HPLC. Methanol was used as the mobile phase, the flow rate was 1 ml/min, a C18 column was used at 30°C, and the absorbance was measured at 325 nm. As modifications from the manual (National Agriculture and Food Research Organization, 2018), the solvent for re-dissolution after solvent removal in the evaporator and the flow rate of mobile phase for HPLC measurement were modified from isopropanol to methanol, and from 2 to 1 mL/min, respectively.

2.5 | Feasibility test of the direct extraction method

Blood collected on the same day from six randomly selected cattle (two cows, one heifer, and three fattening cattle aged 27–28 months) was used to verify the validity of the direct extraction method. The whole blood collected at the Nagasaki Agriculture and Forestry Technical Development Center (Nagasaki, Japan) was sent to the Kyushu Center of AIST (Saga, Japan) under 10°C condition and measured the day after the blood was collected. Blood retinol levels were also measured by standard protocol using HPLC as true value, and the measured value by direct extraction method was compared with the true value.

2.6 | Investigation of the optimal measurement wavelength

The optimal measurement wavelength was investigated in this experiment because it has been reported that the optimal measurement wavelength for vitamin A analysis by the direct fluorescence method is at a higher wavelength than the peak fluorescence wavelength (Watanabe et al., 2008). Blood samples (33) were obtained from 30 cattle. These data were collected on different 4 days. Because blood was taken from randomly selected cattle, three of the 30 cattle were sampled twice during this 4-day period, giving a sample size of 33. The whole blood collected at the Nagasaki Agriculture and Forestry Technical Development Center (Nagasaki, Japan) was measured the day after the blood was collected as well as the prior test. The optimal measurement wavelength obtained in this experiment was reflected in the design of a newly developed portable vitamin A analyzer.

2.7 | Feasibility test of periodic retinol monitoring in fattening cattle using the rapid analysis system

Changes in blood retinol in 12 Japanese Black cattle were measured by the developed rapid analysis system as practical verification. HPLC measurements were also performed to obtain reference data. Blood draws were performed on the same day for all 12 cattle. The average age of the 12 cattle on the day of the first blood draw was 15.5 ± 0.57 months, and the periodic measurement was continued until 26.8 ± 0.57 months old. Whole blood was analyzed at the Nagasaki Agriculture and Forestry Technical Development Center on the same day that the blood was drawn. The 12 cattle were fattened in a free-range barn and were not grazed. During the test period, they were fed only a formula feed and rice straw in a ratio of 100%–110% total digestible nutrients to that of the Japanese Feeding Standard for Beef Cattle, with an expected daily weight gain of 0.8–1.0 kg (National Agriculture and Food Research Organization, 2009). In addition, 125,000 IU of retinol preparation

was orally administered a total of six times at 21.2, 22.1, 23.0, 23.9, 25.7, and 26.6 ± 0.57 months of age.

2.8 | Statistical analysis

In this study, the measurements of the developed method were evaluated by examining the correlation coefficients and their *p* values with the blood retinol concentration measured by the HPLC method. These analyses were performed by using Fit Y by X tool of JMP 14 (SAS Institute Inc., NC, USA).

3 | RESULTS AND DISCUSSION

3.1 | Feasibility test of the direct extraction method

Figure 1 shows the fluorescence spectra of six blood samples pretreated by the direct extraction method and excited at a light wavelength of 330 nm. The fluorescence signal derived from retinol was observed from 400 to 600 nm, with a peak of 470 nm (Figure 1a) (Futterman et al., 1975). The peak fluorescence value between 400 and 600 nm was highly correlated with blood retinol level. The correlation coefficient between blood retinol level and fluorescence signal at 470-nm wavelength was 0.98 (Figure 2b). This experiment demonstrated that the combination of direct extraction from whole blood and fluorescence measurement will detect blood retinol.

3.2 | Investigation of the optimal measurement wavelength

Thirty-three blood samples were pretreated by the direct extraction method and the fluorescence spectra at an excitation light of 330 nm were measured. Pearson's correlation coefficients between blood retinol level obtained by HPLC and fluorescence values at each wavelength were calculated to investigate the optimal wavelength for measurement of blood retinol level (Figure 2a). The correlation coefficients with blood retinol level increased sharply from 410 nm and decreased sharply from 600 nm. High correlation coefficients (>0.77) were observed between 470 and 560 nm. Average fluorescence values at this wavelength band were plotted against blood retinol level (Figure 2b), demonstrating a strong correlation (0.78). The wavelengths of the vitamin A analyzer were determined based on these results.

3.3 | Development of the portable vitamin A analyzer

Only an analyzer that can be brought into the field is needed for vitamin A monitoring. One of the challenges in constructing a small

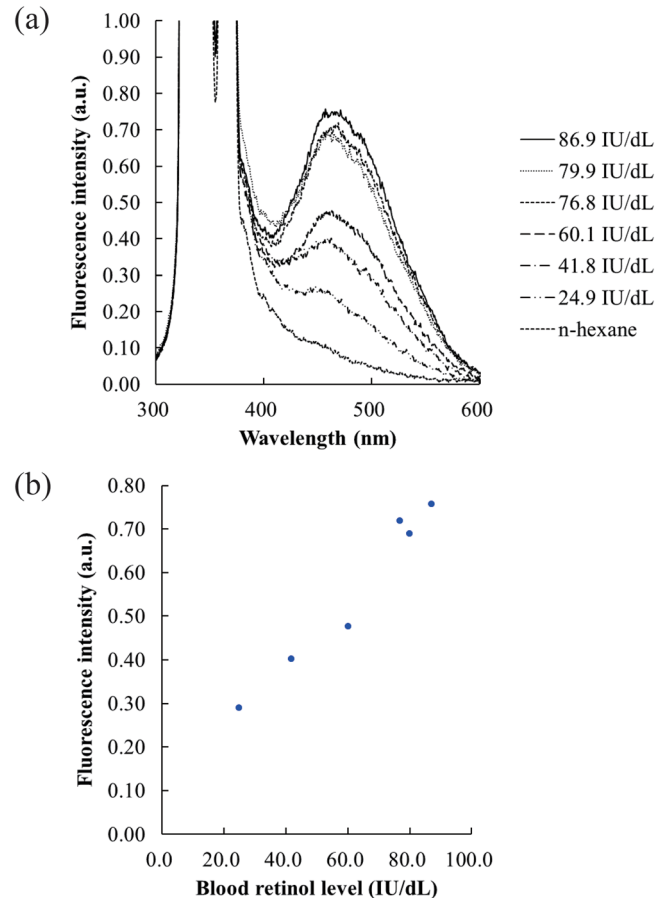


FIGURE 1 Results of fluorescence measurement of six samples extracted directly from whole blood drawn from different six cattle. (a) Fluorescence spectra of hexane and six samples (excitation wavelength is 330 nm). Blood retinol levels of each sample were measured by high-performance liquid chromatography (HPLC). (b) Correlation between the peak fluorescence value between 400 and 600 nm, and blood retinol level

optical system is that it is difficult to protect the robustness of the system because it is susceptible to stray light. Therefore, it is important to take measures against stray light. To resolve this challenge, we developed a compact, inexpensive, sensitive, laser-induced fluorescence (LIF) system (vitamin A analyzer) by using silicone optical technology (SOT) (Nomada et al., 2017) (Figure 3a). The SOT is an optical system composed of a light guide made of silicone resin, with black carbon dispersed around the waveguide to suppress the diffusion of light. Because the noise light entering the black carbon is absorbed by the absorption particles and does not return to the light guide, only the straight light entering from the incident end can be extracted. The vitamin A analyzer consists of a light-emitting diode (LED), a photo-multiplier tube-type photodetector, SOT-based waveguides, a lens, a low-pass filter, a band-pass filter, and a test tube holder (Figure 3b). An LED with a wavelength of 340 nm was used, which is the absorption wavelength band of retinol. The band-pass filter with a central wavelength of 520 nm, and a passband width of 70 nm was used as a band-pass filter, based on the results of the prior investigation

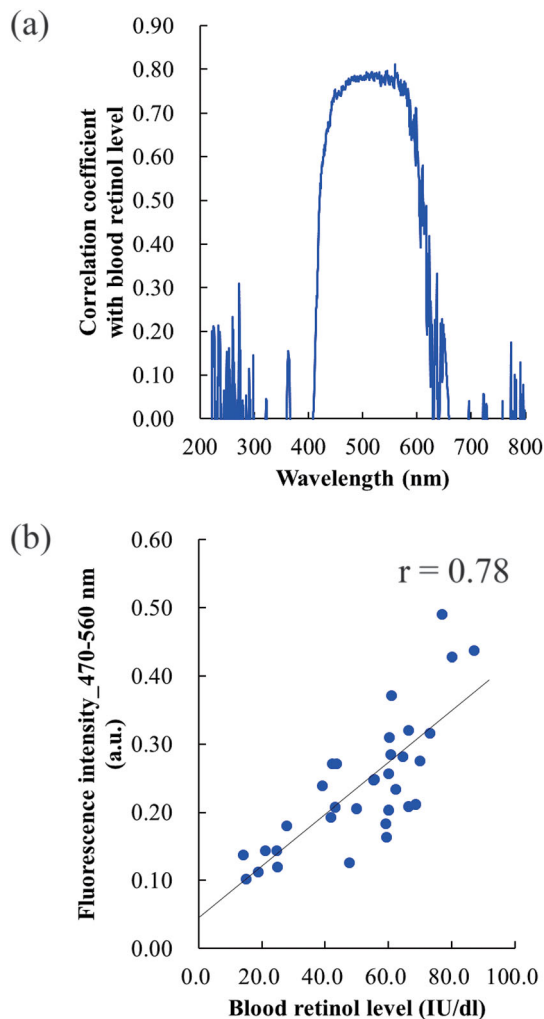


FIGURE 2 Correlation between fluorescence wavelength and blood retinol level measured using a total of 33 blood samples collected from 30 cattle. (a) Correlation coefficients of fluorescence intensity at each wavelength with blood retinol level. (b) Fluorescence intensity in the 470- to 560-nm band at each blood retinol level. The value of vertical axis is the average of the fluorescence intensities in that range. The correlation coefficient between the blood retinol level and the measured values was 0.78 ($p < 0.01$)

experiment. The sample is dispensed in a 10-mm diameter quartz test tube, which is then set in a holder for measurement. The light emitted from the LED is focused by a lens; the wavelength of the detection band is cut off by a low-pass filter, and introduced into the SOT waveguide. It paths through the SOT waveguide, and only the straight light irradiates the test tube. The fluorescence light of retinol paths through a second SOT-based waveguide and band-pass filter, and is detected by the photodetector. This simple vitamin A analyzer can be wirelessly connected to a tablet or mobile to operate and display the results. It is portable because it can be battery powered. This small, inexpensive device can be taken to the production site and be used for on-site evaluation of blood retinol in beef cattle.

To verify the retinol detection capability of this vitamin A analyzer, we measured a standard reagent of retinol dissolved in

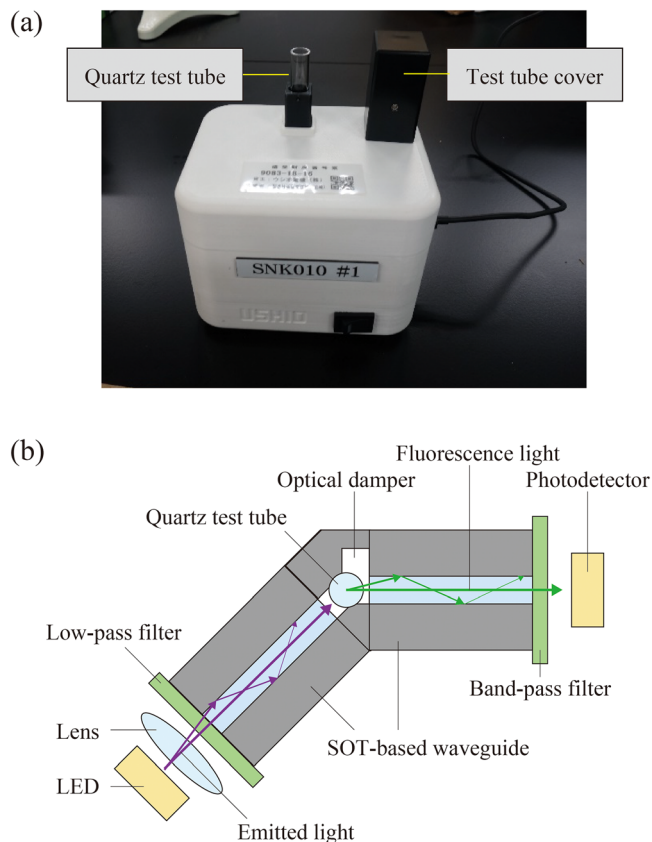


FIGURE 3 Simple vitamin A analyzer. (a) Photograph of the vitamin A analyzer. (b) Schematic of the optical systems for the vitamin A analyzer. LED, light-emitting diode; SOT, silicone optical technology

n-hexane. Retinol was adjusted in concentrations ranging from 1 to 1000 ng/ml. This range is also the concentration range of 0.3 to 300 IU/dl. Figure 4 shows the calibration curve of the retinol standard reagent measured using the vitamin A analyzer. Figure 4b shows a magnified view of the range from 0 to 20 IU/dl. Linearity was obtained in the range of high to low concentrations, and a high correlation (0.9993) was obtained. This result demonstrates the high retinol measuring ability of the vitamin A analyzer.

3.4 | Feasibility test of periodic retinol monitoring in fattening cattle using the rapid analysis system

The measurements of 155 blood samples collected periodically from 12 fattening cattle using the developed simple analysis method and the HPLC method are shown in Figure 5a. In the concentration range of 10 to 100 IU/dl, the vitamin A analyzer measurements fell between 1000 and 1900. This value is comparable with the concentration range of 0 to 15 IU/dl measured using a standard sample. The vitamin A analyzer showed linearity over this range of measurements, which is sufficient for the performance of the device. In the present method, blood and *n*-hexane are mixed at a ratio of 1:10, and retinol is

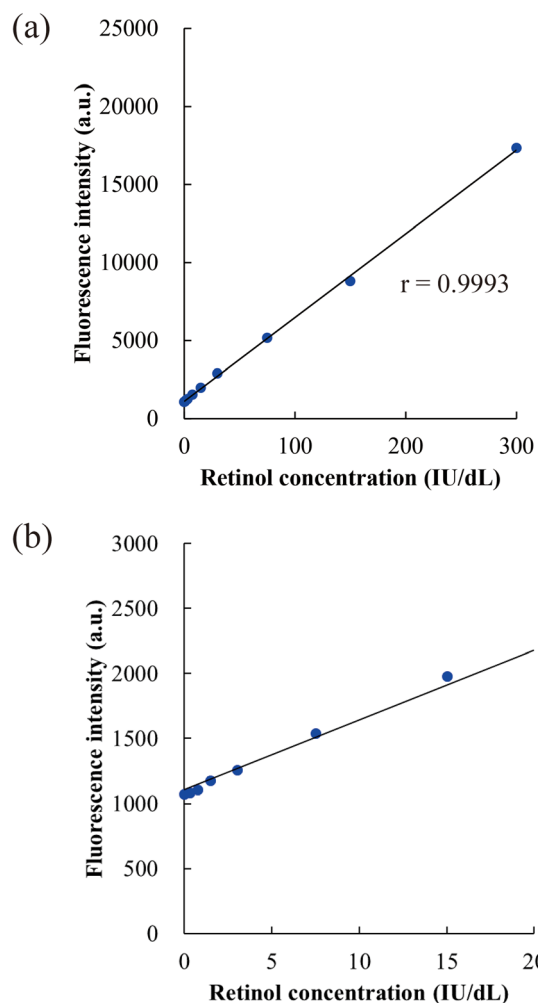


FIGURE 4 Calibration curve of the retinol standard reagent measured using the vitamin A analyzer (a) and the magnified view of the range from 0 to 20 IU/dl (b). A high correlation coefficient was obtained between the retinol concentration and the measured values ($r = 0.9993$; $p < 0.01$)

extracted from blood to *n*-hexane, so it is assumed that the concentration is less than one tenth. On the other hand, samples extracted from whole blood contain more contaminants than standard samples, so noise components are added, and it is thought that a fluorescence signal with a concentration equivalent to 1/6 to 1/7 of the actual blood retinol level are obtained. The value measured using the vitamin A analyzer showed a strong correlation (0.78) to HPLC measured blood retinol, but variability was greater in the region of low blood retinol. This is because measurements after 24 months of age were higher than actual blood retinol levels. When the analysis was restricted to <24-months old cattle, the correlation was higher (0.87). One of the reasons for the high value of the three measurements taken after 24 months of age by the vitamin A analyzer may be the deterioration of the vortex mixer. When the vortex mixer was set to 2000 rpm, the actual frequency was about 1320 rpm after long-term use. Another new vortex mixer vibrated at 1140 rpm with the same settings. Because the amount of extraction by solvent extraction

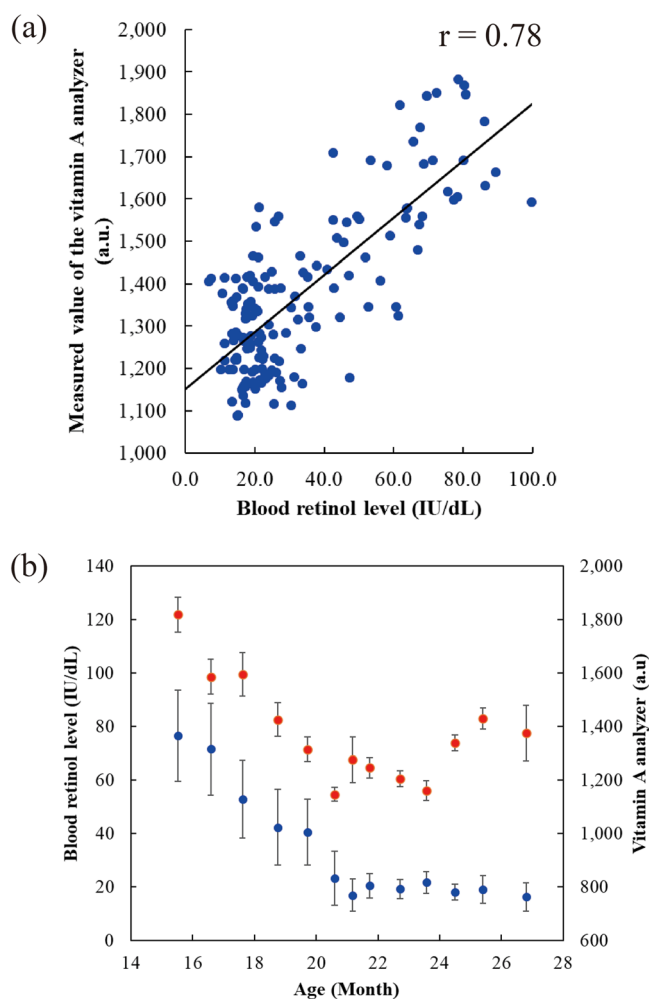


FIGURE 5 Correlation between blood retinol concentrations in a total of 155 samples collected from 12 cattle and the values measured by the vitamin A analyzer (a) fluorescence intensity of 155 obtained from 12 cattle by the direct extraction method with the vitamin A analyzer. The correlation coefficient between the blood retinol level and the measured values was 0.78 ($p < 0.01$). (b) Changes in blood retinol level of 12 cattle measured by HPLC and by the vitamin A analyzer over time. (blue circles); blood retinol level measured by HPLC. (red diamonds); measured value by vitamin A analyzer. Error bars represent standard deviations. The horizontal axis is plotted as the average monthly age of the 12 animals, but actually has a standard deviation of ± 0.57 months

depends on the stirring speed, the stronger vortex oscillation may have increased the amount of extraction and increased the fluorescence intensity. It is not certain when the deterioration of the vortex occurred, but it is possible that failure occurred in measurements after 24 months of age. In terms of the reliability and the robustness of this method for practical use, the relationship between vortex behavior and detection results needs to be investigated in the future.

In addition, other variables such as blood properties should also be considered to improve practicality. For example, it has been reported that the concentration of β -carotene and hemoglobin in blood affects the measured value when their concentration is above

TABLE 1 Correlation coefficients between blood vitamin A level measured by HPLC and the vitamin A analyzer for 12 cattle

No.	Correlation coefficient	
	Until 24 months of age	Entire period
1	0.93	0.85
2	0.86	0.77
3	0.89	0.80
4	0.93	0.88
5	0.96	0.81
6	0.89	0.71
7	0.81	0.74
8	0.91	0.74
9	0.94	0.83
10	0.90	0.78
11	0.88	0.86
12	0.92	0.89
Whole	0.87	0.78

Note: For all correlations, the *p* values were less than 0.01.

400 and 1 mg/ml, respectively, using the direct fluorescence method (Miyamoto et al., 2003). This is because the absorption wavelength band of β -carotene and hemoglobin overlaps with the fluorescence wavelength band of retinol (Suzuki & Katoh, 1990). In this study, the cattle were not grazed and were fed only roughage during the experimental period, so it is thought that β -carotene concentration in blood was kept at low level, but care should be taken, depending on the fattening method. As for hemoglobin, because it is extracted with hexane, the effect is thought to be smaller than when it is measured by diluting it with water, but the effect needs to be investigated.

The correlations between blood retinol and the value obtained with the vitamin A analyzer were better in individual cattle than those overall (Experiment 3). Table 1 shows correlation coefficients for each individual animal. Almost all of the animals showed a high correlation (>0.88) between HPLC and vitamin A analyzer measurements until 24 months of age. Only one animal (No. 7) showed a worse correlation (0.81) than did the others. Blood retinol levels in cattle (other than No. 7) continued to decline until 24 months of age. No. 7 had higher blood retinol at 19.7 and 20.6 months of age (Figure S2), as measured by HPLC, even though the values measured by the vitamin A analyzer continued to fall, as did the other cattle, suggesting that the poor correlation in No. 7 may be due to inaccurate HPLC measurement at 19.7 and 20.6 months of age.

All cattle had blood retinol levels below the danger level of 30 IU/dl after 20 months of age. Even the test sites, which have extensive experience and are thought to be more carefully produced than the average farmer, demonstrate that fattening according to previous rules of thumb results in lower blood retinol than expected. In this experiment, the period of blood retinol deficiency started from the summer season. It is reported that blood retinol levels tend to be lower during the summer season than in other periods, so caution is

warranted (Katamoto et al., 2003). The results also demonstrated that experience alone could not accurately control blood retinol levels, and blood retinol levels need to be constantly monitored in this particular production system.

4 | CONCLUSION

A simple and rapid method for measuring blood retinol levels of cattle in the field was developed. The method is based on a simple pre-treatment of whole blood mixed with normal hexane for 10 min, followed by instantaneous fluorescence measurement with a small, portable, vitamin A analyzer. This method is easy to introduce into the field because it eliminates the cumbersome work of blood cell separation, and allows the whole blood to be used as it is. Better correlation can be obtained by improving the robustness of extraction system. In terms of the reliability and the robustness of the method for practical use, the relationship between the vortex behavior and the detection results needs to be investigated in the future. Samples were vortexed for 10 min, and the mixing ratio of whole blood and *n*-hexane was 1:10, but these parameters should be reconsidered. Refinements for practical use need to be considered. This system enables regular measurement of blood retinol concentrations because it does not require complicated work. Therefore, it is possible to detect trends in changes of blood retinol concentration by regular measurement, even if it includes variations. It is considered that this system is suitable for monitoring the trend in blood retinol concentrations and providing feedback for subsequent fattening methods, rather than evaluating high and low blood retinol concentrations in a single measurement. Whole blood extraction and the developed vitamin A analyzer showed potential to be used for on-farm monitoring of retinol levels in Japanese Black cattle. The relationship between blood retinol levels and meat quality and meat volume has been shown for various breeds of cattle, regardless of whether or not they are Wagyu (Adachi et al., 1999; Arnett et al., 2008; Gorocica-Buenfil, Fluharty, Bohn, et al., 2007; Gorocica-Buenfil, Fluharty, Reynolds, & Loerch, 2007; Oka et al., 1998; Pickworth et al., 2012). Our developed method can be used for these varieties of cattle as well. However, it is considered that this method is particularly effective in high unit price Wagyu cattle that are managed individually using the Japanese method.

CONFLICT OF INTEREST

The authors declare no conflicts of interest associated with this manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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