## Machine Vision Based Prediction of Serum Vitamin A Level in Japanese Black Cattle by Pupillary Light Reflex Analysis

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*Abstract*—To increase the BMS (Beef Marbling Standard) score of Japanese Black Cattle, keeping the cattle serum vitamin A at a low level (30-40 IU/dl) during fattening age is an effective way. The traditional method of monitoring the serum vitamin A level is blood assay. However, it is costly, time-consuming and makes cattle stressful. A new approach by using 2CCD camera is proposed in this study. Pupil reflex of cattle with different vitamin A level was analyzed by image processing.

#### I. INTRODUCTION

High marbling scored beef is the top-ranked beef in Japanese market. To produce beef with high MS (Marbling Score), controlling the serum vitamin A level at a low level (30-40 IU/dl) during fattening age, from 16 to 24 months of age, has been proved to be an effective way [1]-[3]. Nowadays many Japanese black cattle breeders have already adopted this policy. However, it may be dangerous for cattle, because vitamin A deficiency in cattle would increase the probability of catching diseases, such as night blindness, xerophthalmia, or diarrhea[4]-[7]. To keep the cattle healthy, monitoring the serum vitamin A level of cattle frequently during fattening age is critical. Conventional way of testing serum vitamin A level is blood assay, which is invasive, inconvenient, expensive and stressful. Thus, some alternatives which can acquire serum vitamin A level information easily are demanded.

Night blindness is supposed to be the initial symptom of vitamin A deficiency[4]. Night-blinded cattle usually show dilated pupil and no response to light[7]. There is a possible correlation between the pupillary light reflex with vitamin A deficiency. However, there are few researches reported about the relation between serum vitamin A and pupillary light reflex. Matsuda et al. investigated this relation. The result showed there was a negative correlation between the time pupil needed to stop shrinking and serum vitamin A level[8]. Previously, Yoshida began to analyze pupillary light reflex images in 1 second. It was reported that a nonsignificant positive correlation between the time needed to start

shrinking and serum vitamin A level[9]. In this paper, we continued the experiment to confirm the finding.

The main aim of this study is to predict serum vitamin A level in Japanese black cattle by analyzing pupillary light reflex images which acquired by machine vision system and furthermore, build a serum vitamin A monitoring system for Japanese black cattle.

#### II. MATERIALS AND DEVICES

#### A. Materials

This experiment started from December in 2010 to August in 2011 at Hyogo Prefectural Hokubu Agricultural Institute, Japan. At the beginning of this experiment, 24 Japanese black cattle with the ages from 9 to 11 months old and serum vitamin A level from 44.0 to 126 IU/dl were used. Another new group of 18 cattle with the ages from 9 to 11 months old and serum vitamin A level from 32.33 to 73.33 IU/dl were added. The total number of experiment cattle became 42. The pupillary light reflex images in 1 second of the cattle's left or right eye were acquired every month. The conventional blood assay test was also conducted every month before the day on which the images were taken.

#### B. Devices

In this experiment, 2CCD multi-spectral camera AD-080CL (JAI) was used, which can acquire both color images and NIR (Near Infrared) images. The camera has two CCD image sensors to get color and near infrared images. The light through a lens is separated into two light beams by a beam splitter. One of them is focused on a color image sensor and the other is focused on a monochrome image sensor. The color image sensor is a single-chip image sensor with Bayer filters. The monochrome image sensor is equipped with a filter that cuts off the visible light to get a near infrared image of wavelength 750-900nm. The image is 1024 pixels in width and 768 pixels in height. The lens is NT63-240 (Focus Length 12 mm, F 1.8, EDMUND). The camera was combined with two ring-shaped LED lights. One is MDRL-CW50 (MORITEX) white LED light. Polarized (PL) film is installed in front the LED light to reduce the halation. The other is MDRL-CIR31 (MORITEX) NIR LED light, which has a central wavelength of 850 nm. A plastic tube and sponge were installed in front of the LED lights to remove the ambient

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light. The sketch of the device is shown in Fig. 1. Fig. 2 shows the appearance of the device.



Fig. 1. Sketch of experimental device.



Fig. 2. Appearance of experimental device.

### III. METHODS

To obtain high quality images and avoid human error, calibration of setting was performed each time just before the experiment. The setting of system was shown in Table. 1.

I ABLE I
MACHINE VISION SYSTEM SETTING

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-number to 1.8
4	Set shutter speed to 1/30
5	Set master gain to 150
6	Check white balance
7	Take images of Color-Checker

After calibration, the cattle's eyes were covered for two minutes to adapt to the dark environment. The cattle's eyes were covered by the black cloth continuously until the pupillary light reflex images are taken. Then, we adjusted the relative position of camera and the cattle's eye with NIR LEDs. Since cattle are not sensitive to NIR light, the pupil will not shrink at this time. After focused pupil image is shown on the NIR video, the white LEDs were turned on and images were obtained simultaneously. 30 images were recorded in 1 second. Fig.3 shows the sample images.

The images were processed by developed software in Visual Studio 2008 environment and the pupil area



Fig. 3. Color and NIR images of cattle's right eye.

information was obtained.

For the recognition of pupil in color image is easier than in NIR image, the color image is used for the image processing. Procedures of pupil area acquisition were shown in Fig.4. After reading the color image into the memory, the RGB color component image was converted into HSI color space, because there is a remarkable difference between pupil area and non-pupil area in hue value. Hue value was used as the threshold for binarization. The result image is shown in Fig.5 (a). Fig.5 (b) shows the salt and pepper noise is reduced by removing the unconnected points. The pupil area after filling the bright LED area is shown in Fig.5 (c). Noise reduction process was performed again to make the pupil area edge smooth and the final image is shown in Fig.5 (d).







IV. RESULTS AND DISCUSSION

To investigate the relationship between the pupillary light reflex and serum vitamin A level, pupil area, normalized pupil area, starting shrinking time, slope of pupil contraction regression line, and ratio of max length and breadth of pupil before shrinking were evaluated.

#### A. Pupil Area Size

Fig.6 shows the change of pupil area in 1 second after the

white LED light was on. Different curves represent the same cattle's reaction in different months with different serum vitamin A level. When cattle's eye was irradiated by white LED light, the eyeball would move to another direction or the eye would be closed to avoid strong light irradiation. It caused the lack of some month's data.

The starting points of the pupil area in the same cattle were different. One reason is the variances of relative position of the camera and the eye in different months. Position change includes two aspects, angle change and distance change. By experiment, the pupil area error caused by angle change is mainly under 5% of the averaged pupil area. However, slightly change of distance can affect the pupil area greatly. Another reason is the cattle's mood also affects the initial pupil area. So, it is difficult to evaluate the change of pupil area independently.



Fig. 6. Pupil area change in 1 second.

#### B. Normalized Pupil Area Size

Pupil area was normalized by dividing by the initial pupil area. Normalized pupil area change in 1 second is shown in Fig.7. By using normalized pupil area, the change of pupil area can be compared. Fig.7 showed that at the beginning of the pupillary light reflex, the normalized pupil area didn't change. Different serum Vit.A cattle showed different starting time to shrink. Also, the contraction speed varied in different serum vitamin A cattle.



Fig. 7. Normalized pupil area change in 1 second.

#### C. Starting Shrinking Time

Starting shrinking time, which was defined by Yoshida, is the time needed for the normalized pupil area shrunk to 0.98. Yoshida investigated 42 cattle during their fattening phase from 15 to 20 months old. A nonsignificant positive correlation between serum vitamin A level and starting shrinking time was found. The result was shown in Fig.8.

To confirm the relation between vitamin A and starting shrinking time, Yoshida's data were combined with the data of this experiment in Fig.9. The data show the starting shrinking time of cattle both in and out of fattening age do not have correlation with serum vitamin A as the cattle only in fattening age have.



Fig. 8. Relation between serum vitamin A and starting shrinking time of cattle in fattening age. Data was presented by Yoshida (2011)



Fig. 9. Relation between serum vitamin A and starting shrinking time.

#### D. Slope of Pupil Contraction Regression Line

The slope of pupil contraction line can be used to represent the pupil contraction speed. The bigger slope absolute value implies the faster contraction speed relatively. Fig. 10 shows the change of slope of pupil contraction line of the same cattle in different months. The regression line is the single linear regression line of the pupil contraction curve acquired by ordinary least squares method in EXCEL 2007.

Fig. 11 shows the relation between slope of pupil contraction line and serum vitamin A level. For the experiment cattle are still in the early fattening age, there are few specimens in low vitamin A region. The tendency is not obvious.



Fig. 10. Slope of pupil contraction regression line.



Fig. 11. Relation between slope of pupil contraction regression line and serum vitamin A level.

# *E.* Ratio of Max Length and Breadth of Pupil before Shrinking

The pupil contraction process is much like the changing process of round to oval, as shown in Fig. 12. For the variance of the pupil shape in different month, the ratio of the max length (green line in Fig. 12) and breadth (black line in Fig. 12) before the pupil constriction need to be considered when comparing pupillary light reflex.



Fig. 12. Max length and breadth of pupil. Yellow parts represent the pupil area. Green line represent the pupil max length and black line represent the pupil breadth.

In this part, five factors to evaluate pupillary light reflex are introduced. However, it is still difficult to predict the serum vitamin A level by these factors separately. In future study, multiple linear regression method should be used to investigate the linear relation between the serum vitamin A level and the combination of these pupillary light reflex factors. Also, because of individual difference, it is difficult to directly predict the serum vitamin A level. It is more feasible to define several serum vitamin A grades to represent the serum vitamin A status of cattle and study the correlation between these grades and pupillary light reflex.

#### V. CONCLUSION

In this paper, a new method by using machine vision system to predict serum vitamin A level in Japanese black cattle has been present. The relationship of five factors related to pupillary light reflex and serum vitamin A level in Japanese black cattle has been evaluated. Linear correlation between single factor and serum vitamin A level has not been found. It is suggested to use multiple linear regression method to investigate the correlation between pupillary light reflex and serum vitamin A level in future study.

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