Feasibility of pupillary light reflex analysis to identify vitamin A deficiency in Japanese black cattle

Shuqing Han[a*], Naoshi Kondo[a], Yuichi Ogawa[a], Tateshi Fujiura[a], Shinya Tanigawa[a], Tomoo Shiigi[a], Moriyuki Fukushima[b], Namiko Kohama[b], Hyeon Tae Kim[c], Tatsuya Morisako[a]

[a] Graduate School of Agriculture, Kyoto University, Kitashirakawa-Oiwakecho, Sakyo-ku, Kyoto, Japan

[b] Hyogo Prefectural Hokubu Agricultural Institute, Department of Beef Cattle Production, Wadayamacho-Yasui, Asako, Hyogo, Japan

[c] Department of Bio-Industrial Machinery Engineering, Gyeongsang National University (Institute of Agriculture and Life Science), Jinju, Korea

Abstract:

To produce beef with a higher marbling standard, Japanese black cattle farmers usually actively attempt to lower the serum vitamin A level in the fattening period to a minimum desired level (about 30 IU/dL). However, early identification of suboptimal vitamin A deficiency in the cattle is important to prevent them from becoming susceptible to contracting serious diseases. In this study, we investigated the feasibility of using Pupillary Light Reflex (PLR) analysis to identify vitamin A deficient cattle during this fattening period. PLRs of 43 cattle were recorded monthly from June 2012 to February 2013 using a 2CCD camera based handheld machine vision system. A new image processing algorithm to segment the pupil from the background was developed. Compared with manually selected results, the root mean square error associated with the constriction amplitude (*CA*) acquired by the image processing algorithm was only 2.3%, indicating the

effectiveness of this algorithm. No significant differences were found between results of *CA* from fattening cattle in the high (>60 IU/dL) and low (<30 IU/dL) vitamin A period. However, two fattening cattle were identified with severe vitamin A deficiency, because of their weak PLR (*CA*<10%). Initial pupil roundness (*IPR*) results showed cattle in a dark environment possessed less-dilated pupils during the vitamin A deficient period ($p<0.05$). These results highlight the potential, as well as the limitations of this method. Due to the natural variation of PLR in healthy cattle, monthly measurements are not sufficient to make accurate identification. To realize early identification of severe vitamin A deficiency in fattening cattle, a more frequent PLR measurements regime needs to be explored.

*Keywords***:** Machine vision, Image processing, Pupillary light reflex, Vitamin A deficiency, Japanese black cattle, Precision livestock farming

1. Introduction

Marbling -intramuscular fat- is the intermingling or dispersion of fat within the lean muscle tissue of beef cattle. The degree of marbling is the most influential factor in determining beef quality grade in the USA, Japan, and Australia (Strong, 2004), since it is indicative of the expected taste characteristics (tenderness, juiciness and flavor) of the cooked product. In Japan, the Beef Marbling Standard (BMS), established by the Japanese Meat Grading Association, is applied to the grading of beef. Silicon resin models of the BMS are shown in Fig. 1. These are used to standardize the degree of marbling. The No. 12 model indicates the highest marbling standard.

In an attempt to optimize the degree of marbling, Oka (1998) found that maintaining a low serum

vitamin A level in Japanese black cattle during the fattening period, from 15 to 23 months old, could produce beef with significantly higher BMS. Similar experiments conducted by other researchers (Siebert et al., 2006; Gorocica-Buenfil et al., 2007; Kruk et al., 2008) also confirmed this finding. Currently, most Japanese black cattle farmers actively manipulate the vitamin A levels in cattle to ensure beef with a high BMS. An ideal course for serum vitamin A level in cattle during the fattening stage (Hyogo Prefectural Hokubu Agricultural Institute) is shown in Fig. 2. However, maintaining such a low minimum vitamin A level is difficult. Moreover, lowering the vitamin A level too much, below 30 IU/dL, increases the susceptibility of the cattle to diseases, such as night blindness, xerophthalmia, or diarrhea, and even death (Moore, 1941; O'Donoghue, 1955; Millemann et al., 2007; Issi and Gül, 2010). Thus, it is important to monitor changes in vitamin A levels in cattle to assure they remain in a healthy condition during the vitamin A manipulation period. Currently, a blood assay is the conventional way to measure serum vitamin A level during this time. However, it is invasive and stressful to the cattle. Furthermore, this measurement is inconvenient and expensive; many farmers cannot afford it. Thus, an alternative system which can identify cattle with suboptimal vitamin A levels below 30 IU/dL would contribute to a feeding management regime that does not subject the cattle to vitamin A levels below a threshold value.

Early clinical signs of vitamin A deficiency usually include ocular changes, like papilledema, mottling of the tapetum, pallor of the nontapetum, and xerophthalmia (Maggs et al., 2008). Previous studies from our group showed there existed a negative relationship between serum vitamin A level and tapetum reflection (Takahashi et al., 2011), as well as with the red component ratio of cattle pupils (Takao et al., 2011; Han et al., 2013). Pupil color showed the highest

correlation with serum vitamin A level. A serum vitamin A estimation model, with an estimation error of about 10 IU/dL has been developed based on color (Han et al., 2013). To make the identification of vitamin A deficient cattle more reliable, consideration of multiple factors is necessary. In this study, the relation between Pupillary Light Reflex (PLR) and serum vitamin A level was investigated.

O'Donoghue (1955) reported some cattle, which were vitamin A deficient, showed dilated pupils and no response to light. Another report confirmed the loss of PLR in cattle, as seen in clinical signs of vitamin A deficiency (Issi and Gül, 2010). PLR analysis is an indirect measure of neural network function. Some features extracted from PLR, such as initial pupil diameter, constriction latency, constriction amplitude, constriction velocity, and pupil diameter at maximum constriction have been studied in relation to aging (Bitsios et al., 1996; Daluwatte et al., 2012), time-of-day (Yu et al., 2007) and function of melanopsin-containing retinal ganglion cells (Ishikawa et al., 2012) in humans. PLR analysis is a reliable test, and has the potential to be used to assist the diagnosis of eye related diseases.

Speculating that vitamin A deficiency may also affect PLR in cattle, Matsuda et al. (1999) investigated the relation between constriction duration and vitamin A level. Their results showed that cattle with lower vitamin A levels tended to need a longer time to halt constriction. These findings indicate that the potential to use PLR analysis to identify vitamin A deficient cattle. In the experiment Matsuda et al. (1999) conducted, it usually took more than 10 seconds to record the full PLR of cattle. Such a long illumination time could stress the cattle. With considerations for animal welfare and a more rapid identification methodology, a one second PLR was used in this experiment.

In previous work, we reported a new machine vision system based on a 2CCD camera (Han et al., 2011). The handheld machine vision system is capable of capturing images in a full PLR process. Using that machine vision system, we investigated the relation between PLR and serum vitamin A level based on five characteristics: pupil area, normalized pupil area, starting shrinking time, slope of pupil contraction regression line, and the ratio of max length and breadth of pupil before shrinking. No linear correlations between these factors and vitamin A level were, however, found (Han et al., 2011). It must be noted though, in that preliminary experiment, the PLR analysis was not carried out accurately. The pupil area calculation was based on binarization by the hue value, which was calculated from RGB using a HSI color model (Gonzalez and Woods, 2010). The identified pupil area was incorrect when the boundaries of the pupil overlapped with the bright image of the LED ring that was formed by a specular reflection. Such an image of the LED ring is shown in Fig. 4. Especially, after the constriction, the pupil was usually small and frequently overlapped with the image of the LED ring. Therefore, the objective of the present study was to develop a robust image segmentation algorithm to calculate pupil area and to investigate the feasibility of using a one second PLR analysis to identify vitamin A deficient cattle during the vitamin A manipulated fattening stage.

2. Materials and methods

2.1. Cattle

This experiment was conducted from June 2012 to February 2013 at Hyogo Prefectural Hokubu Agricultural Institute, Japan. PLRs of the right eye of 43 Japanese black cattle, including 25 females and 18 males, were recorded once a month. At the beginning of the experiment, the ages

111 of the cattle ranged from 13 to 16 months and vitamin A levels ranged from 38 to 151 IU/dL
112 (mean ± SD 76 ± 18 IU/dL). From April 2012, the cattle were subjected to a low vitamin A diet.
113 The objectives and methods of this study were explained to, and approved by, researchers from
114 Hyogo Prefectural Hokubu Agricultural Institute.
115
116 2.2. Device
117 A 2CCD multi-spectral camera AD-080CL (JAI) installed with a NT63-240 lens (Focus Length 12
118 mm, F 1.8, EDMUND) was used to acquire both color and Near Infrared (NIR) images. The
119 camera was combined with two ring-shaped LED lights: a MDRL-CW50 (MORITEX) white LED
120 light, and a MDRL-CIR31 (MORITEX) NIR LED light with a central wavelength of 850 nm. A
121 plastic tube was installed in front of the LED lights to block out any ambient light. A sketch of the
122 device is shown in Fig. 3. The captured image is 1024 pixels in width and 768 pixels in height.
123
124 2.3. Experimental Procedures
125 The experiments were usually started at 9:30 in the morning and ended at 4:30 in the afternoon.
126 Firstly, the animal's head was fastened to a fence by ropes. Then, the eyes of the cattle were
127 covered by a ordinary black cloth for about two minutes to allow them to adapt to a dark
128 environment. This black cloth cover was kept on during image recording. Before beginning
129 measurements, the light intensity at the end of the plastic tube was set to 1700 lx and the same
130 lighting condition was kept for all measurements. To maintain a constant pupil position throughout
131 recording, the plastic tube in the front of the machine vision system was kept perpendicular to and
132 in contact with the outer surface of the eyelids. The upper and lower eyelids were held open by

133 hand to guarantee the visualization of the pupil. The focus of the camera was adjusted to the
134 cattle's eye with the NIR LED lighting. After a focused image of the pupil was obtained on the
135 NIR video monitor, the white LEDs were turned on and eye images were recorded simultaneously
136 at 30 frames per second. Sample images are shown in Fig. 4. A blood sample was taken one to two
137 days before the image acquisition. Serum vitamin A level in the blood was determined using the
138 High-Performance Liquid Chromatography (HPLC) method of Bieri et al. (1979).
139
140 2.4. Image Processing
141 Due to the reflection of the white LED light from the tapetum lucidum of the cattle, a unique blue
142 color can be seen in the pupil area. It is easier to segment the pupil from the background with
143 these color images than it is with the NIR images, as shown in Fig. 4. Color images were
144 processed by a program developed in Matlab 2007 (Mathworks). The procedure for pupil area
145 acquisition is shown in Fig. 5.
146 The color image was converted from the RGB color space into the HSI color space (Gonzalez and
147 Woods, 2010), which is claimed to be the closest approximation to human interpretation of color.
148 The hue image is shown in Fig. 6 (b). After binarization based on the hue value, as shown in Fig. 6
149 (c), the boundary of the largest region of interest (ROI) was selected, as shown in Fig. 6 (d).
150 Because the pupil was partially overlapped by the image of the LED ring, it was difficult to select
151 the correct pupil area by thresholding, as shown in Fig. 6 (c). Because the pupil of a cow is oval
152 shaped, an ellipse is good for estimation of pupil area. The boundary of the largest ROI, after
153 removing the overlapping LEDs' reflection, as shown in Fig. 6 (e), was used to conduct ellipse
154 fitting based on a least squares method introduced by Rosin (1993). The fitted result is shown in

Fig. 6 (f). The ellipse shown in Fig. 6 (g) was used to represent the pupil area for subsequent PLR analysis. To evaluate the performance of this image algorithm, the pupils in the images were carefully circled by hand in ImageJ (Abràmoff et al., 2004). The total number of pixels inside the circled area was taken as the gold standard.

2.5. Pupillary Light Reflex Analysis

In order to correct for differences in the pupil area between cattle and measurements, all pupil data were normalized by regarding the resting pupil area, the largest pupil area before turning on the white LED light, as a baseline. Once all normalized pupil areas were calculated from the 30 images taken over the 1 second duration, a pupilogram curve was constructed after smoothing by using a Savitzky–Golay filter (Savitzky and Golay, 1964), as shown in Fig. 7. The following parameters were used for subsequent analysis: Constriction Amplitude over 1 second (*CA*), and Initial Pupil Roundness (*IPR*).

After the onset of white LED light, there is latency time of about 0.3 second, as shown in Fig. 7. *CA* over the 1 second was calculated using the following equation (1).

$$CA = \frac{S_0 - S_1}{S_0} \qquad (1)$$

Where s_0 is the resting pupil area and s_1 is the pupil area at the end of 1 second constriction.

IPR was used as an indicator of resting pupil shape. The IPR was calculated using the following equation (2).

$$IPR = \frac{4\pi S}{P^2} \qquad (2)$$

Where *S* is the area of the pupil and *P* is the perimeter of the pupil.

177 2.6. Data Analysis

178 The image processing results were evaluated by root mean square error (RMSE). To study the
179 effect of vitamin A deficiency on PLR, data were investigated by unpaired *t* tests and paired *t* tests
180 to examine differences between the means of low (< 30 IU/dL) and high (> 60 IU/dL) vitamin A
181 level groups for PLR parameters, *CA* and *IPR*.

182

183 **3. Results and Discussion**

184 3.1 Evaluation of image processing

185 Sixty PLR measurements were randomly selected from nine months of experiments conducted
186 between June 2012 to February 2013 on 43 Japanese black cattle. The first and thirtieth images
187 from each measurement were used for comparisons. Pupil area results acquired by image
188 processing were compared with those collected by hand. The correlation coefficient between these
189 methods was 0.99 and 0.95 for pupil area and *CA*, respectively. Root mean square error (RMSE)
190 of pupil area and *CA* was 930 pixels and 2.3%, respectively. As shown in Fig. 8, the results of
191 pupil area and *CA* were normally distributed around the line of equality, which means the results
192 acquired by these two methods were close to each other. Paired *t* tests showed there was no
193 significant difference between the means of these two results ($p>0.05$). This indicates the proposed
194 image processing method is effective at estimating pupil area and *CA*.

195

196 3.2 PLR of low and high serum vitamin A group

197 The serum vitamin A levels in all the cattle gradually declined during the experimental period.
198 Once the vitamin A levels in cattle, as measured by blood assay, were lower than 30 IU/dL, oral

supplementation with vitamin A was conducted. However, the restoration of vitamin A levels usually took weeks. There were many cattle that possessed vitamin A levels lower than 30 IU/dL at the end of the experiment.

3.2.1 *CA* of low and high vitamin A group

Initial pupil shape will have an effect on the *CA*, but to avoid a complicated discussion on this effect, only the *CA* with a *IPR* larger than 0.89, indicating those with a fully dilated resting pupil, were used for subsequent comparisons. Seventy measurements of cattle with vitamin A levels higher than 60 IU/dL were placed in a high vitamin A group. On the other hand, seventy seven measurements of cattle with vitamin A levels under the minimum desired vitamin A level (30 IU/dL) were placed in the low vitamin A group. Their histogram is shown in Fig. 9. Unpaired 2-tailed *t* test showed there was no significant difference between mean *CA*s of the high (mean ± SE 28.8 ± 0.7%) and low (mean ± SE 28 ± 0.8%) vitamin A group (p=0.44). However, the low group showed a larger deviation, which may be caused by vitamin A deficiency. To exclude the effect of individual differences, a paired *t* test was carried out on 30 cattle, which appeared both in the high and low vitamin A groups. A paired 2-tailed *t* test also showed there was no significant difference between the mean *CA*s of the high (mean ± SE 28.3 ± 1.2%) and low (mean ± SE 28.8 ± 1.5%) vitamin A groups (p=0.78). During our experiment two cattle showed a loss of PLR or weak PLR, a result similar to that reported by O'Donoghue and Issi and Gul (O'Donoghue, 1955; Issi and Gül, 2010). *CA*s of these two cattle were less than 10%, as shown in Fig. 9 and Fig. 10. The possible reasons for no significant differences between the mean *CA* of the high and low vitamin A groups might be attributed to a lack of measurements, individual differences between

221 cattle in their tolerance to vitamin A deficiency, natural variation of PLR in healthy cattle, and the
222 effect of unavoidable physical stimulation on PLR during recording.
223
224 Currently, because of the complicated procedures and cost of blood tests, we could only afford to
225 do a monthly PLR analysis. More frequent measurements are necessary to make a clear judgment
226 on the early detection of vitamin A deficiency. Besides that, the pupils were affected by physical
227 stimulation. In this investigation, patting the back of cattle by hand was regarded as a physical
228 stimulation. Six cattle were selected to record their PLRs while continuously patting their back.
229 The results showed clearly that *CA* becomes smaller, as Fig. 11 shows. This is because the
230 emotional status of the cattle (*e.g.*, fear, anger, or excitement), influences the sympathetic nervous
231 system and causes pupillary dilation (Maggs et al., 2008). Even though we tried to keep the cattle
232 calm during recording, the action of holding the upper and lower eyelids of cattle may have
233 affected the PLR results randomly. In the current experiment, only two cattle were successfully
234 identified as severe vitamin A deficiency by PLR analysis, but even two cattle can still cause a
235 significant economic loss to farmers; and also the changes of PLR are difficult to be noticed by
236 farmers during daily feeding. In the next step, to realize early identification and free cattle from
237 stress, an automatic image capturing system installed at the watering place that does not need to
238 fix the head of the cattle in position needs to be appraised.
239
240 3.2.2 *IPR* of low and high vitamin A group
241 An unpaired 2-tailed *t* test showed that *IPR* of the low (mean ± SE 0.888 ± 0.002) vitamin A group
242 was significantly smaller than the high (mean ± SE 0.895 ± 0.001) vitamin A group (p=0.007). To

avoid the effect of individual differences, a paired 2-tailed *t* test was carried out between *IPR* of 34 cattle during the high (mean ± SE 0.898 ± 0.002) and low (mean ± SE 0.886 ± 0.005) vitamin A period. A similar result was obtained ($p=0.013$). Resting pupil size may be related to its' dark adaptation ability. It is possible that when vitamin A deficiency occurred, the dark adaption process become sluggish in cattle, as it does in humans (Kemp et al., 1988). That might explain why there were more less-dilated pupils during the vitamin A deficient stage, as shown in Fig. 12. This result indicates *IPR* may also be used as an indicator of vitamin A deficiency. To our knowledge, this is the first report that vitamin A deficient cattle have smaller resting pupils after a two-minute dark adaption.

4. Conclusions and Future work

An image processing algorithm based on a least squares ellipse fitting was successfully applied to pupil area calculation from images of cattle eyes. The root mean square error of *CA* was only 2.3% compared to the manually selected results, which were taken as the gold standard. A two sample *t*-test and paired *t*-test showed there was no significant difference ($p>0.05$) in *CA* between the low and high vitamin A cattle groups. However, two cattle were identified with weak PLR (*CA*<10%), a result similar to that reported by O'Donoghue, Issi and Gul (O'Donoghue, 1955; Issi and Gül, 2010). It shows that abnormal PLR, which is caused by severe vitamin A deficiency, can be quantitatively assessed by our method. Because of the natural variability in PLR of healthy cattle and the infrequent measurement regime of cattle in this experiment, most cattle with serum vitamin A levels lower than 30 IU/dL were not correctly identified. A more frequent sampling regime is worth testing to see if the effectiveness of this method can be improved. On the other

hand, compared with cattle during the high vitamin A stage, there were more cattle that had smaller resting pupils ($p<0.05$) in a dark environment during the vitamin A deficient stage. *IPR* may also be used as an indicator of vitamin A deficiency. To realize more frequent and economical measurements, an automatic image capturing system installed at the watering place is one suggested solution. *CA* and *IPR* can be analyzed each cattle come to drink water. Besides that, water use of individual cattle can also be recorded. As feeding and watering can be closely related (Kashiha et al., 2013), any reduced drinking activity may indicate anorexia, another early clinical sign of vitamin A deficiency (Issi and Gül, 2010).

Acknowledgements

This study was supported by JSPS KAKENHI Grant Number 23380153. We gratefully acknowledge the financial support of Japanese Ministry of Education, Culture, Sports, Science and Technology. We want to thank the staff of Hyogo Prefectural Hokubu Agricultural Institute for their help and support with the cattle. We are grateful to Professor Garry John Piller (Graduate School of Agriculture, Kyoto University, Japan) and Emeritus Professor Malcolm Fitz-Earle (Capilano University, Canada) for their help in revision of the manuscript.

References

Abràmoff, M.D., Magalhães, P.J., Ram, S.J., 2004. Image processing with imageJ. Biophotonics International 11, 36-41.

Bieri, J.G., Tolliver, T.J., Catignani, G.L., 1979. Simultaneous determination of α-tocopherol and retinol in plasma or red cells by high pressure liquid chromatography. Am. J. Clin. Nutr. 32, 2143-2149.

288 Bitsios, P., Prettyman, R., Szabadi, E., 1996. Changes in autonomic function with age: A study of
289 pupillary kinetics in healthy young and old people. Age Ageing 25, 432-438.
290 Daluwatte, C., Miles, J.H., Christ, S.E., Beversdorf, D.Q., Lofgreen, A., Berliner, N., Yao, G., 2012.
291 Age-dependent pupillary light reflex parameters in children, San Diego, CA, pp. 3776-3779.
292 Gonzalez, R.C., Woods, R.E., 2010. Digital image processing. Pearson Education International, Upper
293 Saddle River, N.J.
294 Gorocica-Buenfil, M.A., Fluharty, F.L., Bohn, T., Schwartz, S.J., Loerch, S.C., 2007. Effect of low
295 vitamin A diets with high-moisture or dry corn on marbling and adipose tissue fatty acid composition
296 of beef steers. J. Anim. Sci. 85, 3355-3366.
297 Han, S., Kondo, N., Fujiura, T., Ogawa, Y., Takao, Y., Tanigawa, S., Fukushima, M., Watanabe, O.,
298 Kohama, N., 2011. Machine vision based prediction of serum vitamin A level in Japanese black
299 cattle by pupillary light reflex analysis, 2011 IEEE/SICE International Symposium on System
300 Integration (SII), pp. 178-181.
301 Han, S., Kondo, N., Ogawa, Y., Mano, S., Takao, Y., Tanigawa, S., Fukushima, M., Watanabe, O.,
302 Kohama, N., Kim, H.T., Fujiura, T., 2013. Estimation of serum vitamin A level by color change of
303 pupil in Japanese black cattle. Engineering in Agriculture, Environment and Food 6, 177-183.
304 Ishikawa, H., Onodera, A., Asakawa, K., Nakadomari, S., Shimizu, K., 2012. Effects of
305 selective-wavelength block filters on pupillary light reflex under red and blue light stimuli. Japanese
306 Journal of Ophthalmology 56, 181-186.
307 Issi, M., Gül, Y., 2010. Case reports: Hypovitaminosis A coupled to epilepsy in four calves. Revue de
308 Medecine Veterinaire 161, 387-390.
309 Kashiha, M., Bahr, C., Haredasht, S.A., Ott, S., Moons, C.P.H., Niewold, T.A., Ödberg, F.O.,

310 Berckmans, D., 2013. The automatic monitoring of pigs water use by cameras. Comput. Electron.
311 Agric. 90, 164-169.
312 Kemp, C.M., Jacobson, S.G., Faulkner, D.J., Walt, R.W., 1988. Visual function and rhodopsin levels in
313 humans with vitamin A deficiency. Experimental Eye Research 46, 185-197.
314 Kruk, Z.A., Bottema, C.D.K., Davis, J.J., Siebert, B.D., Harper, G.S., Di, J., Pitchford, W.S., 2008.
315 Effects of vitamin A on growth performance and carcass quality in steers. Livest. Sci. 119, 12-21.
316 Maggs, D.J., Miller, P.E., Ofri, R., 2008. Slatter's fundamentals of veterinary ophthalmology. Saunders,
317 Philadelphia.
318 Matsuda, K., Watanabe, A., Ichijo, T., Yashima, T., Ujiie, K., Kawana, A., 1999. The relationship
319 between blood vitamin A concentration and oculopupillary reflex in Japanese black fattening cattle. J
320 Vet Clin 47, 239-244. (in Japanese)
321 Millemann, Y., Benoit-Valiergue, H., Bonnin, J.P., Fontaine, J.J., Maillard, R., 2007. Ocular and cardiac
322 malformations associated with maternal hypovitaminosis A in cattle. Vet. Rec. 160, 441-443.
323 Moore, L.A., 1941. Some ocular changes and deficiency manifestations in mature cows fed a ration
324 deficient in vitamin A. Journal of Dairy Science 24, 893-902.
325 O'Donoghue, J.G., 1955. Blindness in beef cattle and its possible relationship to vitamin A deficiency.
326 Canadian Journal of Comparative Medicine 19, 61-64.
327 Oka, A., Maruo, Y., Miki, T., Yamasaki, T., Saito, T., 1998. Influence of vitamin A on the quality of
328 beef from the Tajima strain of Japanese black cattle. Meat Sci. 48, 159-167.
329 Rosin, P.L., 1993. A note on the least squares fitting of ellipses. Pattern Recognition Letters 14,
330 799-808.
331 Savitzky, A., Golay, M.J.E., 1964. Smoothing and differentiation of data by simplified least squares

procedures. Analytical Chemistry 36, 1627-1639.

Siebert, B.D., Kruk, Z.A., Davis, J., Pitchford, W.S., Harper, G.S., Bottema, C.D.K., 2006. Effect of low vitamin A status on fat deposition and fatty acid desaturation in beef cattle. Lipids 41, 365-370.

Strong, J., 2004. Differences in carcass grading schemes used in the USA, Japan and Australia. Aust. J. Exp. Agric. 44, 675-680.

Takahashi, N., Kondo, N., Watanabe, O., Fukushima, M., Iwaki, F., Tuan, N.Q., Yoshida, K., Shiigi, T., Ogawa, Y., Fukuzono, K., Nakano, M., 2011. Effects of serum vitamin a level on tapetum in Japanese black cattle eye. Engineering in Agriculture, Environment and Food 4, 126-130.

Takao, Y., Kondo, N., Ogawa, Y., Han, S., Tanigawa, S., Fujiura, T., Fukushima, M., Watanabe, O., Kohama, N., 2011. Correlation between serum vitamin A level and pupil color of beef cattle, 2011 IEEE/SICE International Symposium on System Integration (SII), pp. 182-186.

Yu, M., Kautz, M.A., Thomas, M.L., Johnson, D., Hotchkiss, E.R., Russo, M.B., 2007. Operational implications of varying ambient light levels and time-of-day effects on saccadic velocity and pupillary light reflex. Ophthalmic Physiol. Opt. 27, 130-141.

Figure captions

Fig. 1. Silicone resin models used for the evaluation of beef marbling developed by the Japanese National Institute of Animal Industry.

Fig. 2. Ideal serum vitamin A levels 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).

Fig. 3. Sketch of experimental device.

Fig. 4. Color and NIR images of cattle's right eye.

Fig. 5. Pupil area acquisition procedure.

Fig. 6. (a) Original color image, (b) Hue image, (c) Binarized image by hue value,(d) Color image with boundary of largest region of interest shown in red, (e) Color image with boundary of pupil selected for fitting shown in yellow, (f) Fitted ellipse based on least squares method with its long and short axis shown in red, (g) Fitted ellipse used to represent pupil area.

Fig. 7. A pupilogram of cattle's right eye in response to white LED light stimulation with light intensity of 1700 lx.

Fig. 8. Pupil area (a) and *CA* (b) acquired by manually and image processing.

Fig. 9. Histogram of *CA* from high (>60 IU/dL) and low (<30 IU/dL) vitamin A group cattle. Two cattle with CA less than 10% were recognized as loss of PLR and weak PLR, respectively.

Fig. 10. Examples of Loss of PLR, Weak PLR and Normal PLR.

Fig. 11. *CA*s from six cattle's PLRs recorded normally and with intentional physical stimulation.

376

377 Fig. 12. Relationship between *IPR*s and vitamin A levels.

378

Highlights:

- An image segmentation method to calculate pupil sizes of cattle was developed.
- Two cattle were identified as severe vitamin A deficiency by our method.
- Cattle had smaller resting pupil in the dark during vitamin A deficient stage.

Fig. 1

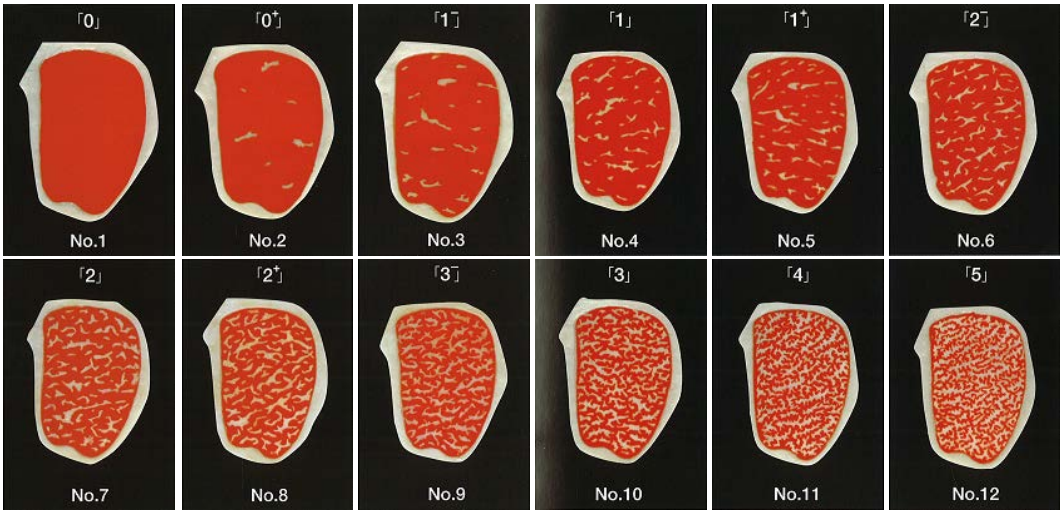

「0」
No.1
「0+」
No.2
「1-」
No.3
「1」
No.4
「1+」
No.5
「2-」
No.6
「2」
No.7
「2+」
No.8
「3-」
No.9
「3」
No.10
「4」
No.11
「5」
No.12

Fig. 2

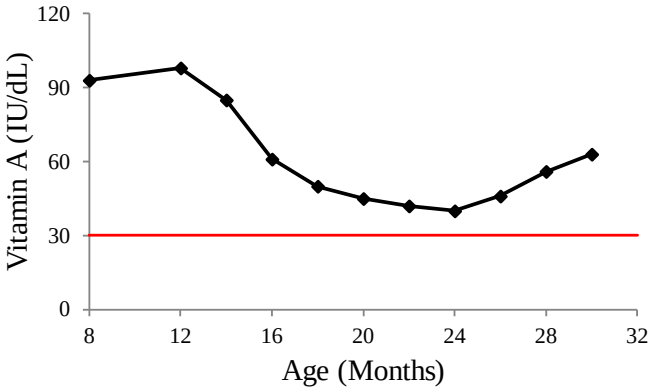


Fig. 3

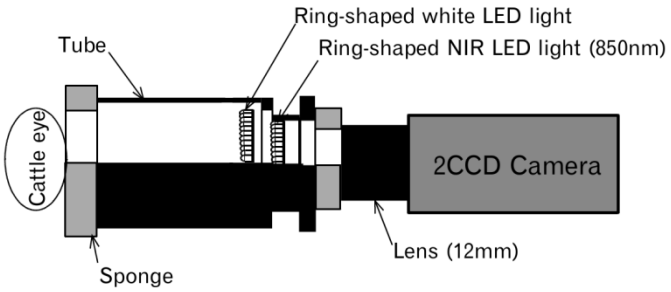

Ring-shaped white LED light
Tube
Ring-shaped NIR LED light (850nm)
Cattle eye
2CCD Camera
Lens (12mm)
Sponge

Fig. 4

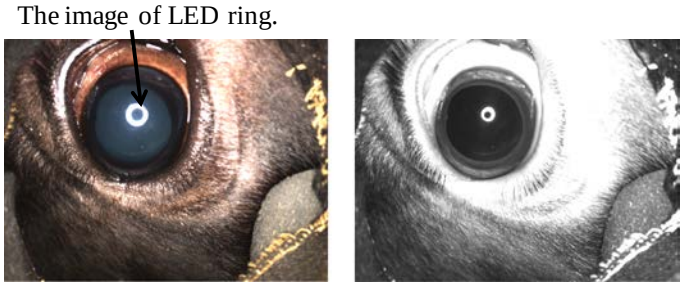


Fig. 5

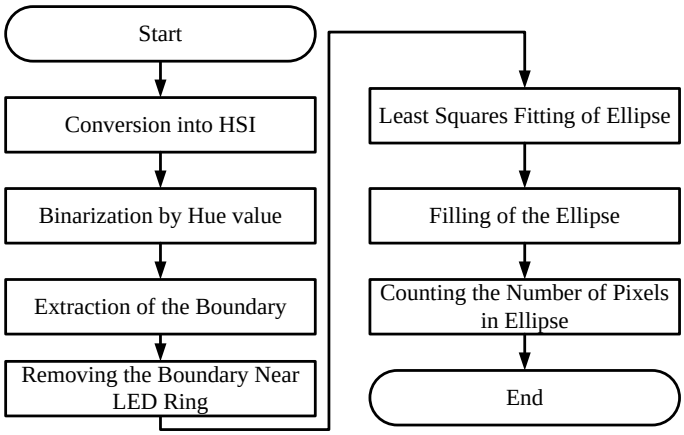

Start
Conversion into HSI
Binarization by Hue value
Extraction of the Boundary
Removing the Boundary Near LED Ring
Least Squares Fitting of Ellipse
Filling of the Ellipse
Counting the Number of Pixels in Ellipse
End

Fig. 6

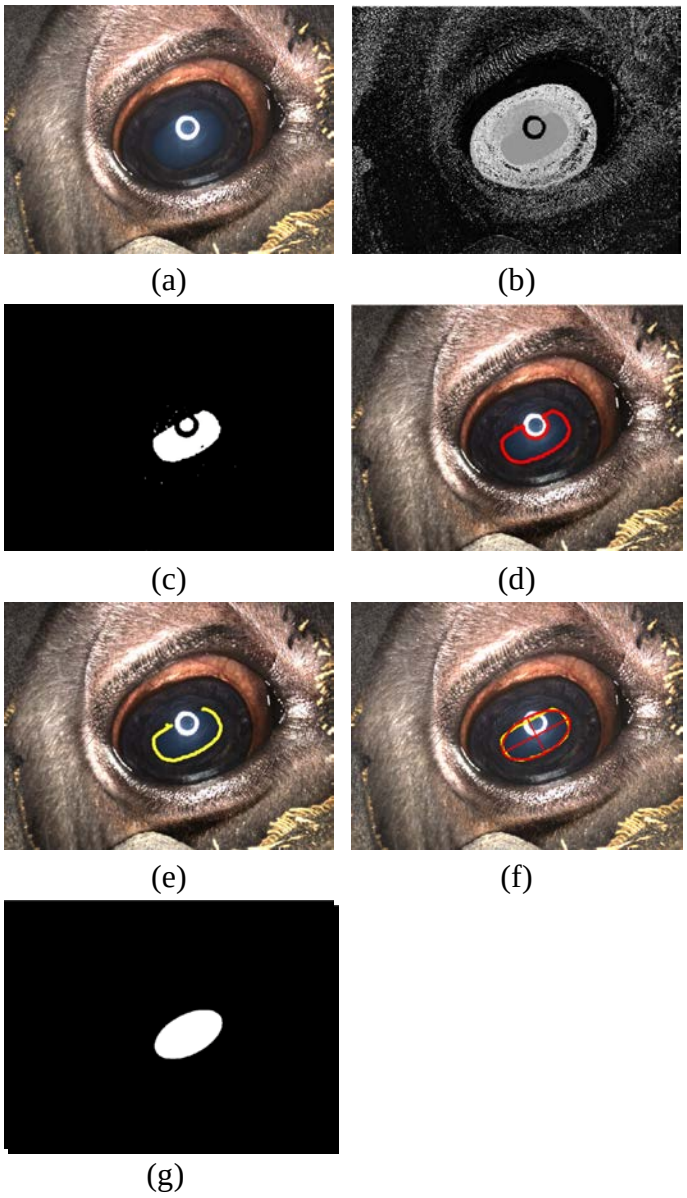


Fig. 7

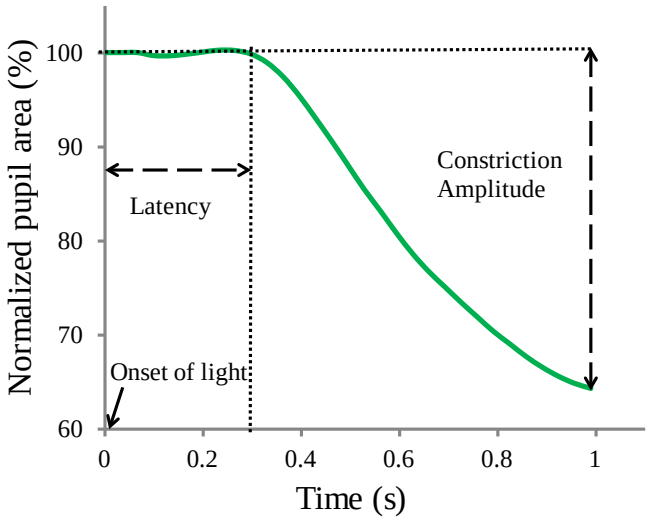
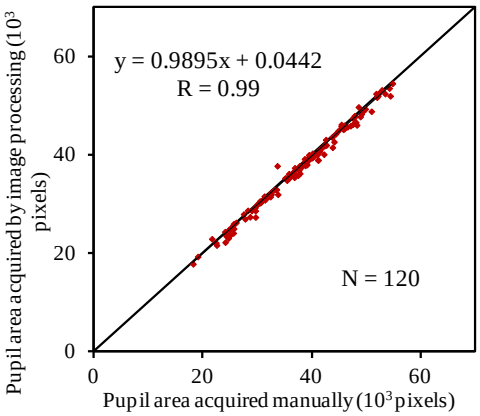
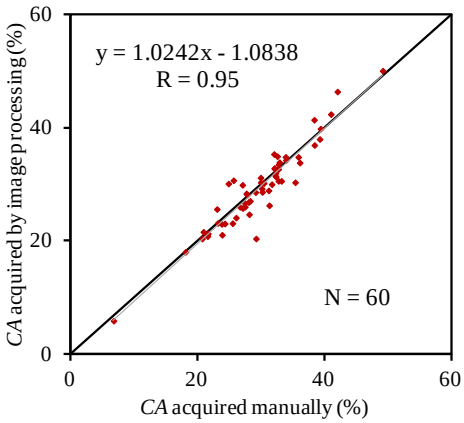

Normalized pupil area (%)
100
90
80
70
60
0
0.2
0.4
0.6
0.8
1
Time (s)
Latency
Constriction
Amplitude
Onset of light

Fig. 8


y = 0.9895x + 0.0442
R = 0.99
N = 120
Pupil area acquired by image processing (10^3 pixels)
Pupil area acquired manually (10^3 pixels)
0
20
40
60

(a)


y = 1.0242x - 1.0838
R = 0.95
N = 60
CA acquired by image processing (%)
CA acquired manually (%)
0
20
40
60

(b)

Fig. 9

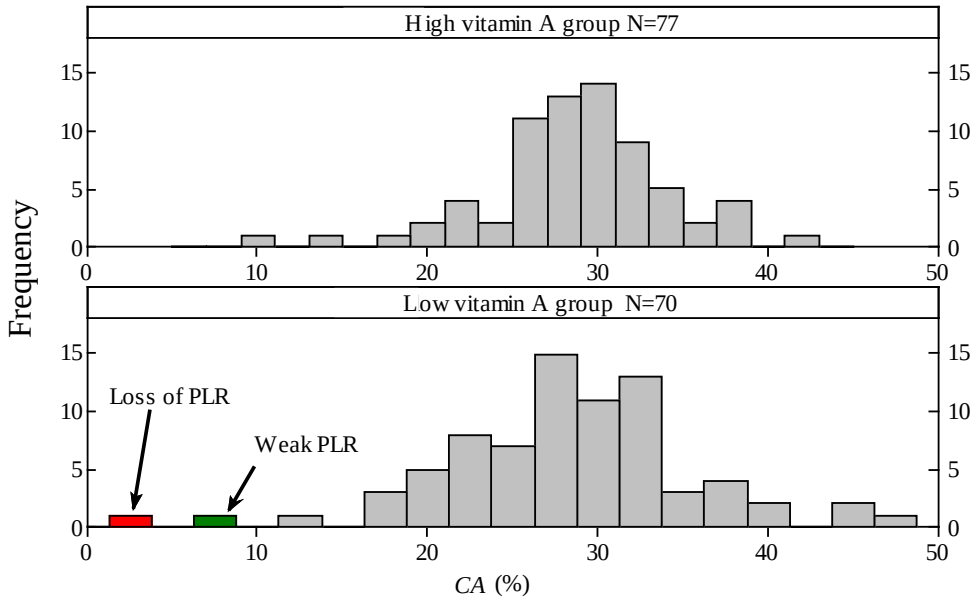

High vitamin A group N=77
Low vitamin A group N=70
Frequency
Loss of PLR
Weak PLR
CA (%)
0
5
10
15
20
30
40
50

Fig. 10

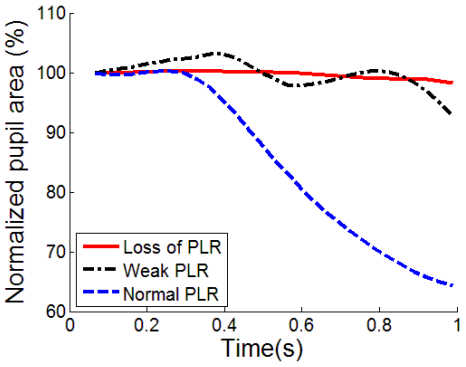

Normalized pupil area (%)
110
100
90
80
70
60
0
0.2
0.4
0.6
0.8
1
Time(s)
Loss of PLR
Weak PLR
Normal PLR

Fig. 11

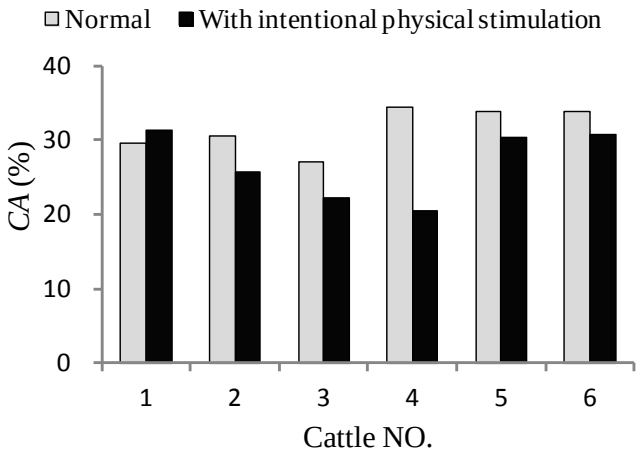
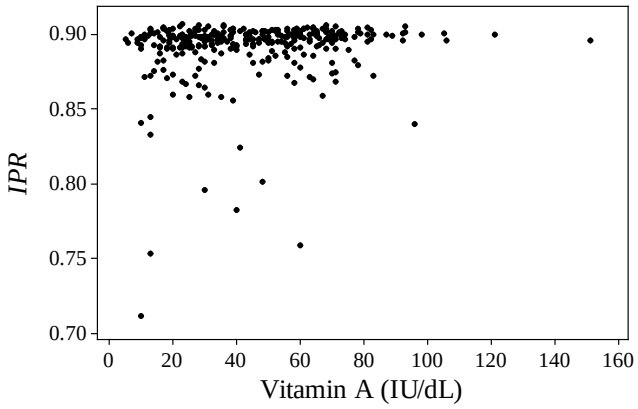

Normal
With intentional physical stimulation
40
30
20
10
0
CA (%)
1
2
3
4
5
6
Cattle NO.

Fig. 12


0.90
0.85
0.80
0.75
0.70
IPR
0
20
40
60
80
100
120
140
160
Vitamin A (IU/dL)