Effects of Low Serum Vitamin A Level on Pupillary Light Reflex in Japanese Black Cattle

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Abstract: Four parameters of pupillary light reflex were investigated from May to December in 2011 to study the effects of low serum vitamin A level on pupillary light reflex in Japanese black cattle during their vitamin A controlled stage. A 2CCD camera was used to acquire the pupillary light reflex images. Constriction amplitude in 1 second, maximum constriction velocity, maximum velocity time and initial pupil roundness were used for pupillary light reflex analysis. Cattle in low vitamin A level period had larger constriction amplitude in 1 second (p=0.001), higher maximum constriction velocity (p=0.016) and thinner resting pupil (p=0.003). This result showed pupillary light reflex analysis can be used as indicators for cattle management during vitamin A controlled stage.

Keywords: Machine vision, Beef cattle, 2CCD camera, Pupillary light reflex, Image processing.

1. INTRODUCTION

To produce beef with high Beef Marbling Standard (BMS), maintaining the serum vitamin A at a low level (30-40 IU/dL) during fattening age, from 16 to 24 months old, has been proved to be an effective way (Oka *et al.* 1998; Siebert *et al.* 2006; Gorocica-Buenfil *et al.* 2007; Kruk *et al.* 2008). Most Japanese black cattle breeders adopt the vitamin A controlling method, as shown in Fig.1. However, it is difficult to keep the cattle at the critical low level. If vitamin A deficiency happened, cattle would be vulnerable to catch diseases such as night blindness, xerophthalmia, or diarrhea, even death (Moore 1941; O'Donghue 1955; Millemann *et al.* 2007; Issi and Gül 2010). It is important to monitor the vitamin A level to keep the cattle healthy and prevent economic loss for the breeders during vitamin A controlled period. Blood assay is the typical way to measure the serum vitamin A level. However, it is invasive and stressful to the cattle. Furthermore, it is complicated and needs expensive devices, high-performance liquid chromatography (HPLC) system which cannot be afforded by most breeders. Thus, an alternative that can estimate vitamin A level easily is desired.

Pupillary Light Reflex (PLR) is an indirect measure of neural network function. Its features such as initial pupil diameter, constriction latency, constriction amplitude, constriction velocity and pupil diameter at max constriction have been studied for aging effects (Bitsios *et al.* 1996; Daluwatte *et al.* 2012), time-of-day effects (Yu *et al.* 2007) and functions of melanopsin-containing retinal ganglion cells (Ishikawa *et al.* 2012). It is a reliable test and has the potential to be used to assist the diagnosis of eye related diseases.

O'Donghue (1955) reported some cattle which were vitamin A deficient showed dilated pupil and no response to light. Another similar clinical sign of vitamin A deficiency was reported by Issi and Gul (2010) that the vitamin A deficient cattle lost PLR. Matsuda *et al.* (1999) investigated the relation between PLR and vitamin A level. The result showed cattle with lower vitamin A level tended to need longer time to stop shrinking. These findings showed that PLR could be used as an indicator of vitamin A deficiency.

A new machine vision system based on 2CCD camera was introduced by us (Han *et al.* 2011). The PLR images of cattle can be acquired by that system. We investigated the relation between PLR and serum vitamin A level from five aspects 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. The linear correlation between these factors and vitamin A level was not found. There were some shortcomings of that experiment. First, cattle's vitamin A levels during the investigation were high, from 73±17 IU/dL at the beginning of the experiment to 49±13 IU/dL at the end of the experiment. According to Japan Livestock Technology Association, 80 IU/dL vitamin A level is necessary to keep cattle healthy and 30 IU/dL is the critical level to keep cattle alive. It is possible that all the cattle in the experiment showed no signs of vitamin A deficiency. Second, the pupil area selection was based on binarization by hue value. The calculated pupil area was incorrect when the pupil was partially covered by eyelid. Third, the parameters used for PLR analysis were not selected properly, e.g. the variation of pupil area in different months was large and the slope of pupil contraction

regression line may not effective to represent the constriction velocity. The aim of present study is to improve the PLR analysis and investigate effects of low serum vitamin A level on PLR in Japanese black cattle.

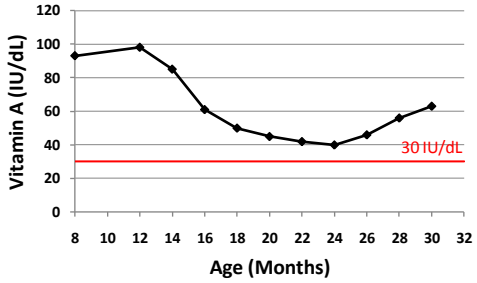

Fig. 1 Desired serum vitamin A level in cattle at different age of growth during fattening stage. The red line indicates the critical level (30 IU/dL) of vitamin A in Japanese black cattle. For vitamin A, 1 IU is the biological equivalent of 0.3 μg retinol, or of 0.6 μg beta-carotene. (Provided by Hyogo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

2. MATERIALS AND METHODS

2.1 Cattle

This experiment was conducted from May 2011 to December 2011 at Hyogo Prefectural Hokubu Agricultural Institute, Japan. Right eye PLR images of 16 Japanese black cattle were taken once every month. The cattle's ages were 16 months and vitamin A level ranged from 37 to 74 IU/dL (mean level 56 ± 10 IU/dL) at the beginning of the experiment. The cattle were subjected to a vitamin A free-diet beginning in April 2011. All the cattle used in this study were clinically healthy during experiment period.

2.2 Device

A 2CCD multi-spectral camera AD-080CL (JAI) was used to acquire both color images and Near Infrared (NIR) images. The lens was a NT63-240 (Focus Length 12 mm, F 1.8, EDMUND). 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. A plastic tube was installed in front of the LED lights to block the ambient light. The sketch of the device is shown in Fig. 2.

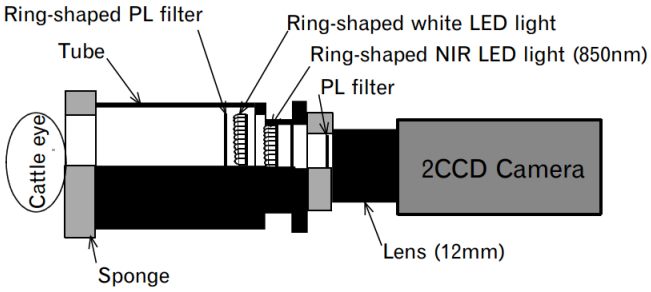

Fig. 2 Sketch of experimental device.

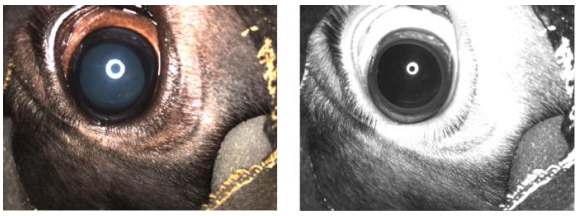

Fig. 3. Color and NIR images of cattle's right eye.

2.3 Experiment Procedures

The cattle' eyes were covered for two minutes to adapt to the dark environment. The cattle's eyes were covered by a black cloth until the eye images were captured. At the beginning of experiment, we set the light intensity to 1700lx to keep the same lighting condition in every experiment. We adjusted the camera position according to the cattle's eye with NIR LEDs on. After focused pupil image was shown on the NIR video, the white LEDs were turned on and eye images were obtained simultaneously. Sample images are shown in Fig. 3. The blood assay was conducted one day or two days before the image acquisition process. The vitamin A level was determined by HPLC.

3. IMAGE PROCESSING & PLR ANALYSIS

3.1 Image Processing

Because of the unique blue color of pupil, it is easier to distinguish pupil from background in color images than NIR images, as shown in Fig. 3. Color images were processed by developed program in Matlab 2007 (Mathworks). Procedures of pupil area acquisition are shown in Fig. 4.

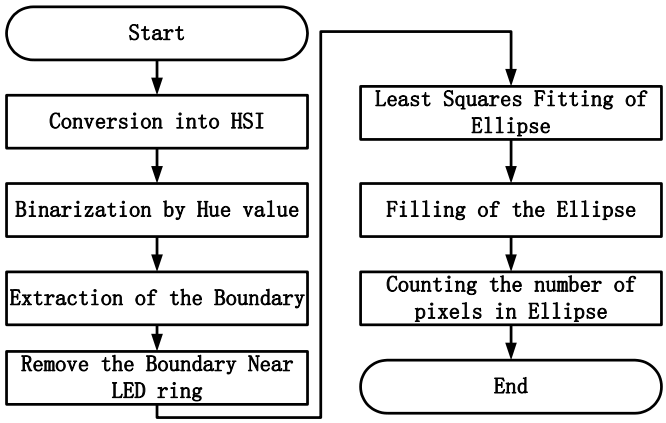

Fig. 4. Pupil area size acquisition procedures.

The color image was first converted into HSI color model (Gonzalez 1992). The hue image is shown in Fig. 5 (b). After binarization based on hue value, the boundary of the largest region of interest (ROI) was selected, as shown in Fig. 5 (d). Because LEDs' reflection was strong, some parts of pupil image were saturated. It was difficult to select the correct pupil area by thresholding, as shown in Fig.5 (c). The boundary of largest ROI after removing near LEDs' reflection part, as shown in Fig. 5 (e) was used to do ellipse fitting based on least squares method. The fitted result is shown in Fig.5 (f). The ellipse shown in Fig.5 (g) was used to represent the pupil area for further PLR analysis.

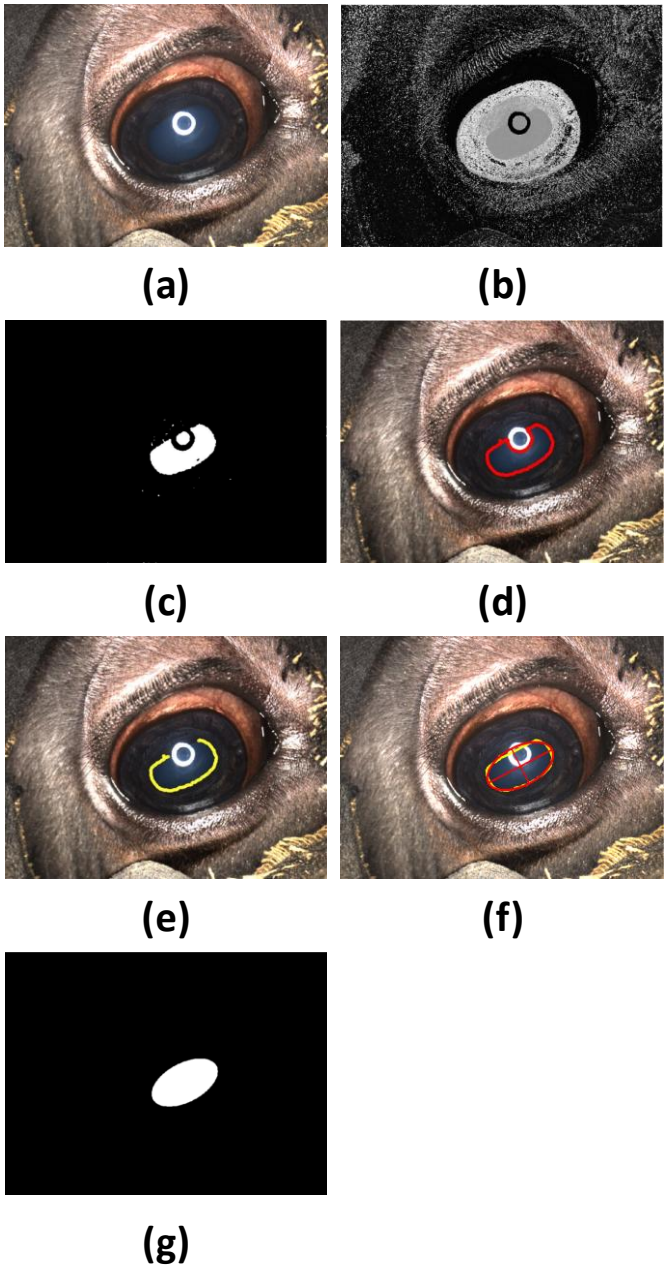

Fig. 5. (a) Original color image, (b) Hue image, (c) Binarized image by hue value,(d) Color image with boundary of largest region of interest shown in red, (e) Color image with boundary of pupil selected for fitting shown in yellow, (f) Fitted ellipse based on least squared method with its long and short axis shown in red, (g) Fitted ellipse used to represent pupil area.

3.2 PLR Analysis

Once all pupil areas were acquired from 30 images taken in 1 second, a pupilogram curve was constructed to represent the pupil area change in response to white LED light stimulus, as shown in Fig. 6. The pupilogram was normalized against resting pupil area to remove effects of resting pupil area when calculating constriction amplitude. The resting pupil area is the pupil area before onset of white LED light. The following parameters were used for PLR analysis, Constriction Amplitude in 1 second (*CA*), Maximum Constriction Velocity (*MCV*), Maximum Velocity Time (*MVT*) and Initial Pupil Roundness (*IPR*).

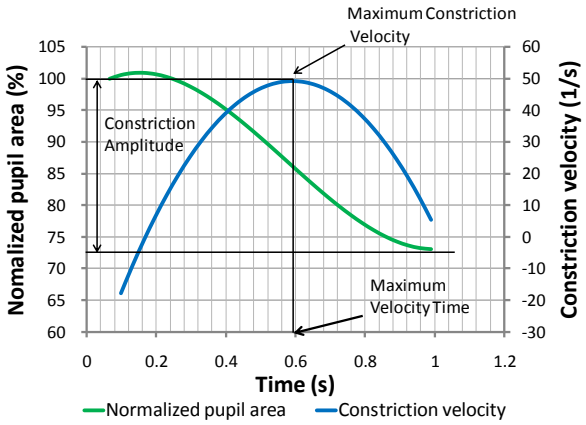

Fig. 6. An illustration of the pupilogram which is normalized against the pupil area before onset of light, shown in green. The instantaneous constriction velocity was shown in blue. Following extracted PLR parameters are shown: Constriction Amplitude, Maximum Constriction Velocity and Maximum Velocity Time.

After the onset of white LED light, the pupil slightly dilated because of light stimulation. The constriction velocity value was minus accordingly. After that pupil constriction started, as shown in Fig. 6. *CA* in 1 second was calculated by (1) below.

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

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

Constriction velocity is the first derivative of the pupilogram curve. *MVT* is the time when *MCV* happens.

IPR was used as the indicator of resting pupil shape.

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

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

4. RESULTS AND DISCUSSION

All the cattle's vitamin A level gradually declined during the experiment period, as shown in Fig. 7. The average vitamin A levels of October (mean level 24 ± 4 IU/dL), November (mean level 23 ± 4 IU/dL) and December (mean level 22 ± 4 IU/dL) were lower than the critical level 30 IU/dL. PLR parameters' average values in these 3 months were considered to represent PLR features of low vitamin A cattle. On the contrary, the average values in May, June and July were used to represent PLR features of high vitamin A cattle. The age effects on PLR were not considered in this paper.

Paired *t* tests showed that cattle in low vitamin A period showed significant larger *CA* (p=0.001), higher *MCV* (p=0.016) and smaller *IPR* (p=0.003). There was no significant difference for *MVT* between low and high vitamin period (p=0.13), as shown in Fig. 8. As the frame rate of used 2CCD camera was 30 frames per second, the time resolution

of current *MVT* measurement is 33ms. This limitation of time resolution restricted the effectiveness of MVT. MVT should be investigated further by using high-speed camera. Table 1 shows the average and standard deviation of cattle PLR parameters in high and low vitamin A period.

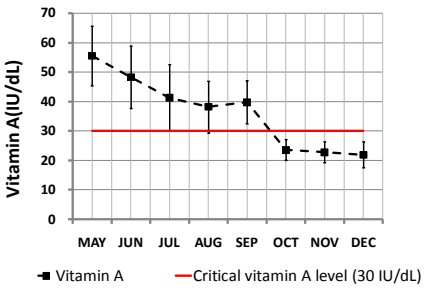

Fig. 7. Time series data of vitamin A from May to December 2011. Error bars indicate the standard deviation of mean.

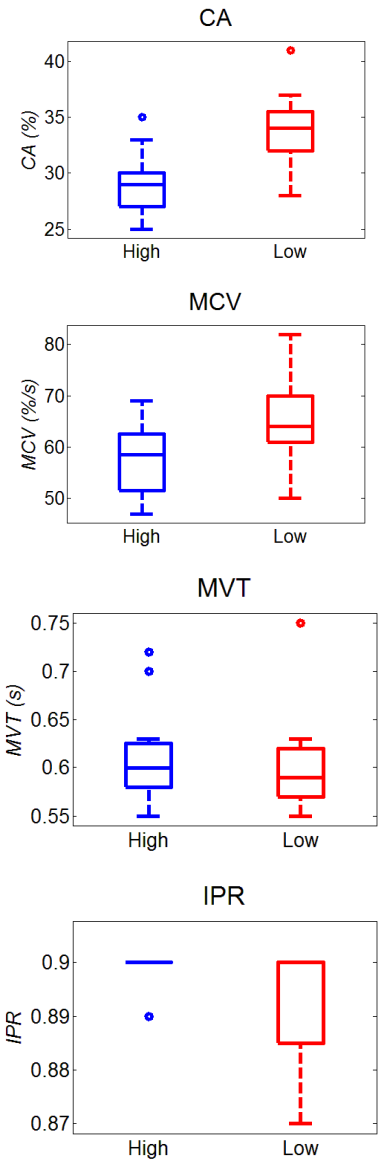

Fig. 8. Comparison of the parameters of PLR between low (in red) and high (in blue) vitamin A level cattle. The horizontal lines are group means, the 'boxes' are SD, the 'whiskers' are extreme values in each group.

Table 1. PLR parameters of cattle in high and low vitamin period

	CA (%)	*MCV* (%/s)	*MVT* (s)	*IPR*
High	29±3	57±7	0.61±0.04	0.90±0.00
Low	34±3	65±8	0.60±0.05	0.89±0.01

Note: Data was based on 1 second PLR test under 1700lx light intensity. mean±std.

Larger *CA* in low vitamin A cattle can be caused by larger *MCV* as Fig. 8 shows and shorter constriction latency, as reported by (Yoshida *et al.* 2011). Constriction latency is the time elapsed between the onset of white LED light and onset of PLR. In cattle, its value was between 0.2-0.4 seconds after onset of white LED light as Fig. 6 shows. For the limitation of time resolution of current system, the constriction latency was not calculated. It should be studied further.

Higher *MCV* was shown when the vitamin A levels were lower, as shown in Fig. 8. PLR analysis was based on 30 images in 1 second, because a fast analysis is necessary for practical application of vitamin A estimation. This result could not compare with previous PLR study of (Matsuda 1999), which based on PLR of more than 10 seconds.

Unlike circular pupils of human, cattle's pupil is oval after constriction. The pupil will change from round shape to slit shape, as shown in Fig. 9. *IPR* can be used as indicator of pupil's opening scale.

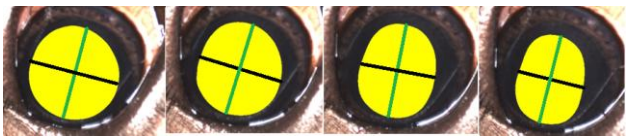

Fig. 9. Change of pupil shape after onset of white LED light. Yellow parts represent the pupil area. Green line represents the pupil max length and black line represents the pupil breadth.

Compared with the cattle with high vitamin A levels, there were many cattle with lower vitamin A levels showed smaller *IPR*, as shown in Fig. 8, which means thinner resting pupil. The relationship between serum vitamin A level and initial pupil roundness were shown in Fig. 10. Resting pupil represents a balance between two antagonistic forces: (1) the amount of incident light stimulating the retina and (2) the emotional status of the cattle (e.g., fear, anger, or excitement), which influences the sympathetic system and causes pupillary dilation (David J. Maggs 2008). As we tried not to excite the cattle during experiment and gently took the PLR images, the thinner resting pupil may be caused by the change of retina sensitivity after vitamin A deficiency. However, to make the mechanism clear, further study is needed.

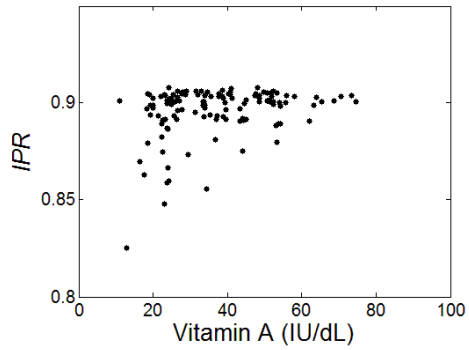

Fig. 10. Relationship between vitamin A level and IPR.

To avoid the discussion of effects of resting pupil shape on *CA* and *MCV*, 90 measurements with *IPR* >= 0.9, which indicate fully dilated pupil after dark adaption, were selected from 128 measurements. Relationship between vitamin A level and *CA*, *MCV* of this group were shown in Fig. 11. Despite the individual variation of PLR and different tolerance of vitamin A deficiency, the negative tendency between *CA*, *MCV* and vitamin A level were clearly shown.

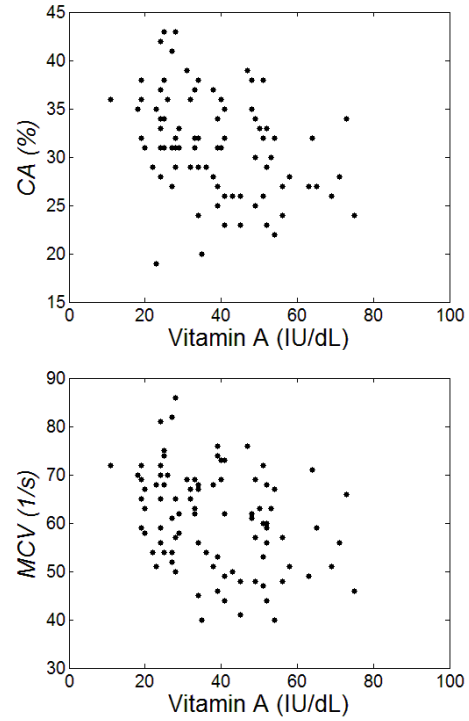

Fig. 11. Relationship between vitamin A level and CA, MCV.

This experiment based on the subjective notion that vitamin A is the main impact factor of PLR. To exclude the effect of age on PLR, another vitamin sufficient control group should be introduced in future study.

5. CONCLUSION

Japanese black cattle in low vitamin A level period showed significant larger constriction amplitude in 1 second, higher maximum constriction velocity and significant smaller initial roundness value. These pupillary light reflex related parameters can be used as indicators of vitamin A deficiency during the vitamin A controlled stage. This information is valuable for beef cattle breeders.

ACKNOWLEDGMENT

This study was supported by Grants-in-Aid for Scientific Research (23380153). We gratefully acknowledge the financial support of Ministry of Education, Culture, Sports, Science and Technology. We also want to thank the staff of Hyogo Prefectural Hokubu Agricultural Institute for their help and support with the cattle.

REFERENCES

Bitsios, P., Prettyman R. and Szabadi E. (1996). Changes in autonomic function with age: A study of pupillary kinetics in healthy young and old people. *Age and Ageing*, 25(6), 432-438.

Daluwatte, C., Miles J. H., Christ S. E., Beversdorf D. Q., Lofgreen A., Berliner N. and Yao G. (2012). Age-dependent pupillary light reflex parameters in children. In *Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society*, 3776-3779. San Diego, CA.

Gonzalez, R. C. W. and Richard E. (1992). *Digital image processing.* Addison-Wesley. Reading, Mass.

Gorocica-Buenfil, M. A., Fluharty F. L., Bohn T., Schwartz S. J. and Loerch S. C. (2007). Effect of low vitamin A diets with high-moisture or dry corn on marbling and adipose tissue fatty acid composition of beef steers. *Journal of Animal Science*, 85(12), 3355-3366.

Han, S., Kondo N., Fujiura T., Ogawa Y., Takao Y., Tanigawa S., Fukushima M., Watanabe O. and Kohama N. (2011). Machine vision based prediction of serum vitamin A level in Japanese Black Cattle by pupillary light reflex analysis. *2011 IEEE/SICE International Symposium on System Integration (SII)*, 178-181. Kyoto.

Ishikawa, H., Onodera A., Asakawa K., Nakadomari S. and Shimizu K. (2012). Effects of selective-wavelength block filters on pupillary light reflex under red and blue light stimuli. *Japanese Journal of Ophthalmology*, 56(2), 181-186.

Issi, M. and Gül Y. (2010). Case reports: Hypovitaminosis A coupled to epilepsy in four calves. *Revue de Medecine Veterinaire*, 161(8-9), 387-390.

Kruk, Z. A., Bottema C. D. K., Davis J. J., Siebert B. D., Harper G. S., Di J. and Pitchford W. S. (2008). Effects of vitamin A on growth performance and carcass quality in steers. *Livestock Science*, 119(1-3), 12-21.

Maggs, D. J., Miller, P. E. and Ofri, R. (2008). *Slatter's Fundamentals of Veterinary Ophthalmology*. 320. Saunders Elsevier . St. Louis.

Matsuda, K., Watanabe, A., Ichijo, T., Yashima, T., Ujiie, K., Kawana, A. (1999). The Relationship between Blood Vitamin A Concentration and Oculopupillary Reflex in Japanese Black Fattening Cattle. *Journal of veterinary clinic*, 47(4), 239-244. (In Japanese)

Millemann, Y., Benoit-Valiergue H., Bonnin J. P., Fontaine J. J. and Maillard R. (2007). Ocular and cardiac malformations associated with maternal hypovitaminosis A in cattle. *Veterinary Record*, 160(13), 441-443.

Moore, L. A. (1941). Some Ocular Changes and Deficiency Manifestations in Mature Cows Fed a Ration Deficient in Vitamin A. *Journal of Dairy Science*, 24(10), 893-902.

O'Donghue, J. G. (1955). Blindness in beef cattle and its possible relationship to vitamin A deficiency. *Canadian Journal of Comparative Medicine*, 19(2), 61-64.

Oka, A., Maruo Y., Miki T., Yamasaki T. and Saito T. (1998). Influence of vitamin A on the quality of beef from the Tajima strain of Japanese black cattle. *Meat Science*, 48(1-2), 159-167.

Siebert, B. D., Kruk Z. A., Davis J., Pitchford W. S., Harper G. S. and Bottema C. D. K. (2006). Effect of low vitamin A status on fat deposition and fatty acid desaturation in beef cattle. *Lipids*, 41(4), 365-370.

Yoshida, K., Mano S., Han S., Shiigi T., Yamamoto K., Ogawa Y., Kondo N., Sugimoto C., Fukushima M., Watanabe O. and Kohama N. (2011). Estimation of serum Vitamin A level by pupillary light reflex using visible and near infrared images. *Journal of Kansai Branch of the Japanese Society of Agricultural Machinery*, 110, 59. (In Japanese)

Yu, M., Kautz M. A., Thomas M. L., Johnson D., Hotchkiss E. R. and Russo M. B. (2007). Operational implications of varying ambient light levels and time-of-day effects on saccadic velocity and pupillary light reflex. *Ophthalmic and Physiological Optics*, 27(2), 130-141.