Towards a new measurement method for urine puddle volume in dairy cow houses using fluorescence

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Abstract

Drainage of urine from concrete floors in dairy housing reduces the ammonia emission. Surface area of a urine puddle and puddle depth are among the five most determining parameters predicting ammonia emission. However a validated method to estimate the amount of urine present on the floor at any time to characterize the drainage process of urine in practice is lacking. We propose a new method to estimate puddle volume based on measuring intensity of emitted light from a simulated urine puddle with a known concentration of soluble fluorescing tracer after exposure with light of an appropriate wavelength. To develop this new method an installation was constructed consisting of blue and white LED lights, a 1.4 MP 14-bit CCD camera and different filters. Calibrations of pixel size and flat field corrections were performed. Essential for this approach is an accurate calibration line between the amount of tracer and the intensity of emitted fluorescence light for use in field measurements. Objective of the research presented here was to establish a calibration line for different combinations of tracer concentration and layer thicknesses under controlled conditions. According to theoretical background a linear relationship between amount of a fluorescence tracer present and the fluorescence light intensity could be established.

Keywords: Fluorescence tracer, urine puddle, depth, ammonia emission, dairy.

1. Introduction

Ammonia (NH_3) is one of the nitrogen (N) substances emitted by livestock farming. It leads to environmental problems like acidification and eutrophication and disturbance of N-cycles of ecosystems. The two main sources of ammonia emission from dairy housing are the urine puddles on the walking floor and the slurry in the pits. To meet EU emission ceiling regulation, Dutch government introduced a system of ammonia emission factors for different housing systems. Dutch emission factors for dairy housing systems are partly based on full-scale field measurements according to a measurement protocol (Ogink et al., 2013) and partly based on calculations with an ammonia emission model named ‘Snelstal’ described by Monteny et al. (1998). A sensitivity analysis of the floor compartment of this model by Snoek et al., (2014a) showed that surface area, depth, urea concentration, pH and temperature of a urine puddle are the most important model parameters influencing the ammonia emission. Urea concentration determines the source strength of the puddle and is influenced by the feed ration and water intake of the cows. Surface area and depth of a urine puddle determine the total amount of urea present on a floor and are influenced by the floor characteristics. Puddle temperature and pH results from ambient circumstances and chemical processes taking place in the puddle. Drainage of the urine to an underlying pit reduces the amount of urea available for emission from the floor and is the main reduction principle behind the majority of the mitigation systems in applied in The Netherlands. The only common way at the moment to evaluate mitigation systems is to measure ammonia emissions at full scale. However, this is time consuming and costly, especially for dairy housing because these are almost always naturally ventilated. An emission factor based on model calculations fed with measured model parameters would make development and assessment of mitigation systems faster and less costly. However, validated methods to measure model parameter puddle volume are not available yet. The method describe by Snoek et al. (2010) is one of the first to research the drainage characteristic of floor elements under laboratory conditions. Snoek et al (2014b) and Snoek et al (2015) presented two separated methods to measure urine puddle size and puddle depth in practice. However a validated method to estimate the amount of urine present on the floor at any time to characterize the drainage process of urine in practice is lacking. We propose a new method to estimate puddle volume based on measuring intensity of emitted light from a simulated urine puddle with a known concentration of soluble fluorescing tracer after exposure with light of an appropriate wavelength. Objective of the research presented here was to establish a calibration line for different combinations of tracer concentration and layer thicknesses under controlled conditions.

2. Materials and Methods

The experimental setup is based on the installation described by Snoek et al (2010) (see Figure 1). It consists of a concrete floor element (1) that can be replaced and on which an aluminum tray (2) of 1.4 meter × 1.2 meter can be placed. This tray can also be placed under the concrete floor on a balance scale to weigh the run of from the floor. On this tray or directly on the concrete floor there is the possibility to place different PVC-sheets (3) necessary to perform

different calibrations. Above this platform a 6×6 matrix of blue and white LEDs (4) is mounted at a height of around 2 meters above the concrete floor. These LEDS emit light that is necessary to capture colour pictures or to excite the fluorescence tracer of the concrete floor or in the aluminum tray. Central in this matrix a filter wheel (5) is mounted containing several optical filters to filter reflected light. This light is captured by a 1.4 MP (1040×1392) 14 bit A/D converter CCD camera (6). This camera is housed (7) on top of the matrix together with power equipment for the LED matrix and a computer controlling the filter wheel and camera. The combination of LEDs, camera and filter wheel (4-7) were developed and manufactured by PhenoVation BV.

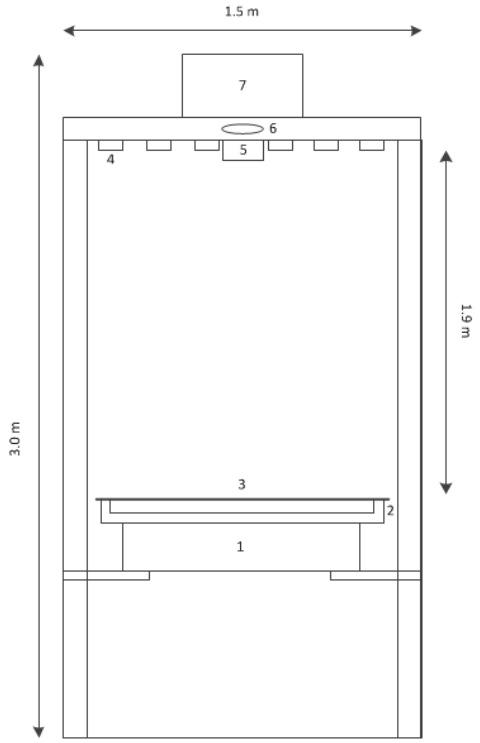

Figure 1. Experimental setup

Fluorescein sodium (CAS number 2321-07-5) was chosen as tracer substance. Excitation wavelength of 485 nm and emission wavelength of 511 nm have been determined before the measurements using a Perkin-Elmer LS55 spectrometer.

According to Aeby et al. (2001) the fluorescence intensity captured by the camera is linear related to amount of tracer in a known volume, given an incident light intensity as long as tracer concentration and layer thickness do not exceed certain values. Knowing this relation and given a known tracer concentration, the puddle volume on the floor can be calculated. The puddle size can be calculated counting the number of exposed pixels and the represented area per pixel. Puddle depth can be calculated from puddle volume and size. Individual pixels are identified by coordinates (x,y).

Final fluorescence intensity image (F) is based on several images and corrections. These images were processed using tracer data analysis software developed by PhenoVation BV. This includes flat field correction image (I) for non-uniform lighting, reflection images (R) for corrections for reflection, background fluorescence image (F_{backgr}) for background correction and a correction for pixel size (lens distortion). Corrections were made according to Aeby et al. (2001).

2.1. Correction for light reflection

Reflection depends on floor material properties and position on the measuring plane. The blue excitation light (c) and the green emission light (t) will reflect differently. Reflection coefficients were calculated as:

$$R_c(x,y) = \frac{I_{floor\ c}(x,y)}{I_{white\ plate\ c}(x,y)} \quad (1)$$

$$R_t(x,y) = \frac{I_{floor\ t}(x,y)}{I_{white\ plate\ t}(x,y)} \quad (2)$$

$$R_{tot}(x,y) = (1 + R_c(x,y)) \times (1 + R_t(x,y)) \quad (3)$$

R is the reflection factor per pixel (x,y) for both blue (c) and green (t) light and the combination of both (tot). I_{floor} is the light intensity of the floor for each pixel (x,y) both for blue (c) and green (t) light and $I_{white\ plate}$ is the light intensity of a white PVC plate which represents 100% reflection for each pixel (x,y) both for blue (c) and green (t) light.

2.2. Background correction

Floor materials itself can contain fluorescing elements or remains of earlier tracer measurements. Therefore each image should be corrected for background fluorescence. A preliminary fluorescence image should be taken before applying tracer. The final image corrected for reflection and background is given by:

$$F_{corrected}(x,y) = \frac{F_{raw}(x,y) - F_{backgr}(x,y)}{R_{tot}(x,y)} \quad (4)$$

F represents the image containing. The background picture without tracers is subtracted from the picture with tracer (raw).

2.3. Pixel size calibration

To minimize Barrel distortion of the camera lens and to assure that each pixel represents an equal area a correction model proposed by Kang et al (2008) was added to the software. Lens correction is dependent on the distance between the camera and the measuring plane.

2.4. Flat field correction

Spatial non-uniform lighting is corrected using an image I(x,y) taken from a fluorescing PVC sheet.

$$F_{final}(x,y) = \frac{F_{corrected}(x,y) \times \bar{I}}{I(x,y)} \quad (5)$$

2.5. Light intensity mass calibration

To calculate the total amount of tracer from the final fluorescence image (F_{final}) a calibration curve is needed that describes the relation between amount of traces and light intensity. To establish such a calibration line an experiment with five different tracer concentrations and nine layer thicknesses was designed using the experimental set up described earlier. Fluorescein sodium solutions of 0.8, 1.7, 2.5, 3.2 and 3.9 mg per litre demineralised water were prepared. To reduce surface tension Agral Gold was added as wetting agent. No fluorescence effect of Agral Gold was found in a preliminary test in a spectrometer at concentration far beyond those used in the experiment. Four petri dishes (90 mm × 14.2 mm) were places right under the camera. Per measuring session one concentration was used. Before filling, images for correction and background fluorescence were taken. A bottle top dispenser (Varispenser) of 5 ml (± 0.005 ml) was used to fill the petri dishes in nine times to a maximum of 45 ml. Each filling represents an average increase of depth of 0.86 mm. After each filling an image was taken. In each session an extra petri dish with a constant amount of tracer solution was measured acting as a reference to be able to correct results for photo bleaching. Fluorescence light intensity is represented as a figure between 0 and 65536 (2^{16}). Combination of incidence light intensity and exposure time was chosen by doing, aiming at use of the full fluorescence light intensity scale for F_{raw} without pixel overexposure. Incident light intensity was set at 50% of full capacity for both white and blue light. Exposure time for white light was 200 μs and for blue light 5,000 μs.

3. Results and Discussion

The fluorescing light intensity of the reference petri dish did always differ less than 0.8% compared with the first measurement of a session. A possible photo bleaching effect is therefore further ignored. The relation between the

fluorescence light intensity averaged over the four petri dishes and the amount of fluorescein for different concentrations is given in Figure 2. It clearly shows that the lines deviate from a linear relation. Moreover at higher volumes a same amount of fluorescein at different combinations of concentration and depth does not result in an equal fluorescence light intensity. As an example in Figure 3 the F_{final} images of measurement 7 (35 ml) using 1.7 mg l^{-1} fluorescein solution is given. The non-linearity and the different light intensities at equal amounts of fluorescein can have following causes:

- The presence of some small bubbles probably caused by the added Agral caused an uneven pattern of light intensity.
- Although the petri dishes were sanded the edges of the petri dishes caused undesirable reflections.
- The fluorescein solution is a clear and yellowish fluid but at higher amount the transparency may be reduced.
- The use of Agral also may lead to a reduced transparency at higher depths.

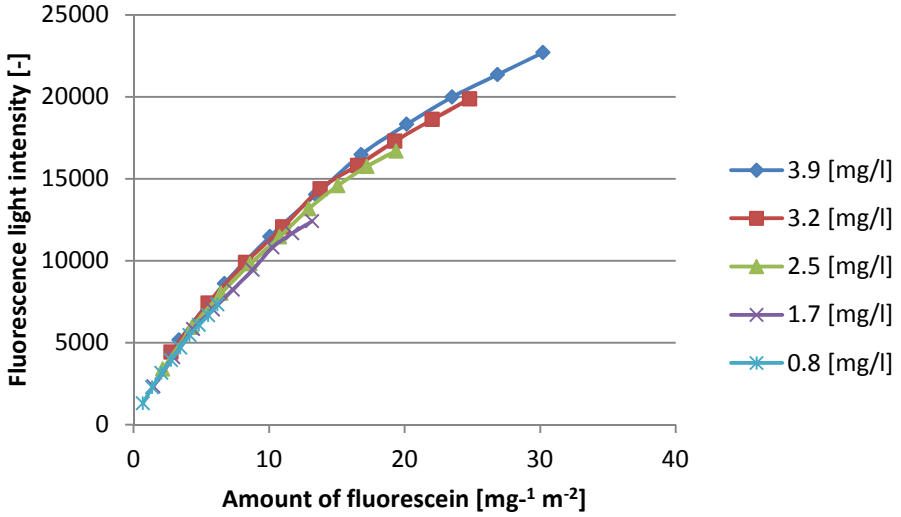

Figure 2. Relationship between amount of fluorescein and fluorescence light intensity (F_{final}) for different fluorescein concentrations

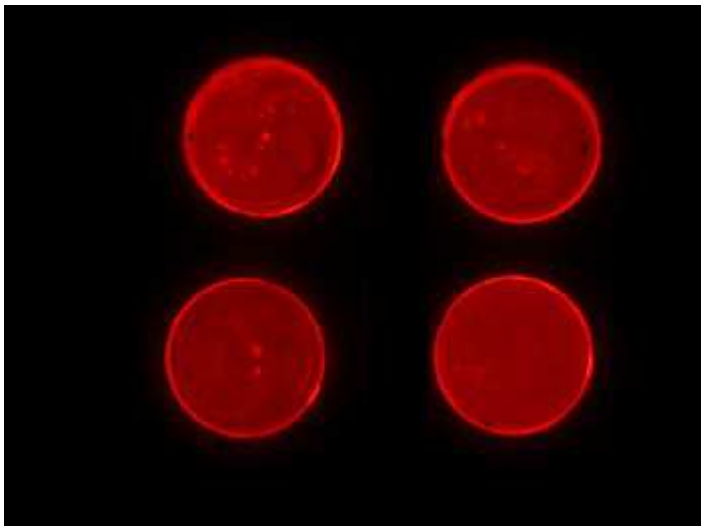

Figure 3. F_{final} of measurement 7 at 1.7 mg^{-1} l^{-1} fluorescein

To see if expected linear relation between amount of fluorescein and fluorescence light intensity occurs when possible disturbing factors are eliminated the described experiment was repeated after some changes:

- Five flat rubber seals places on a black PVC sheet were used instead of petri dishes. Again, one was used as a reference to check for photo bleaching.
- Only concentration of 2.5 mg fluorescein per litre water was used
- No Agral was used as wetting agent.
- A bottle top dispenser (Varispenser) of 0-10 ml (±0.01 ml) was set at 1.10 ml.
- Exposure time for blue light was increased to 40,000 µs.

At the start of the experiment, before taking the background image F_{backgr} two of the four seals were filled with six times 1.10 ml of fluorescein solution to make sure that the whole area enclosed by the seal was covered with fluorescein solution. The experiment commenced by adding an extra 1.10 ml to all seals and taking an image (F_{raw}). A seal was full after ten additions. Each addition represents an average depth increase of 0.5 mm in the seal. After each addition an image was captured. The experiment was repeated twice (A and B). Result are presented in Figure 4. Relation of combined results of both experiments between fluorescence light intensity (y) and amount of fluorescein (x) appeared to be linear this time (R^2=0.99) and can be described by equation 6.

$$y = 1830 \times x - 503 \quad (6)$$

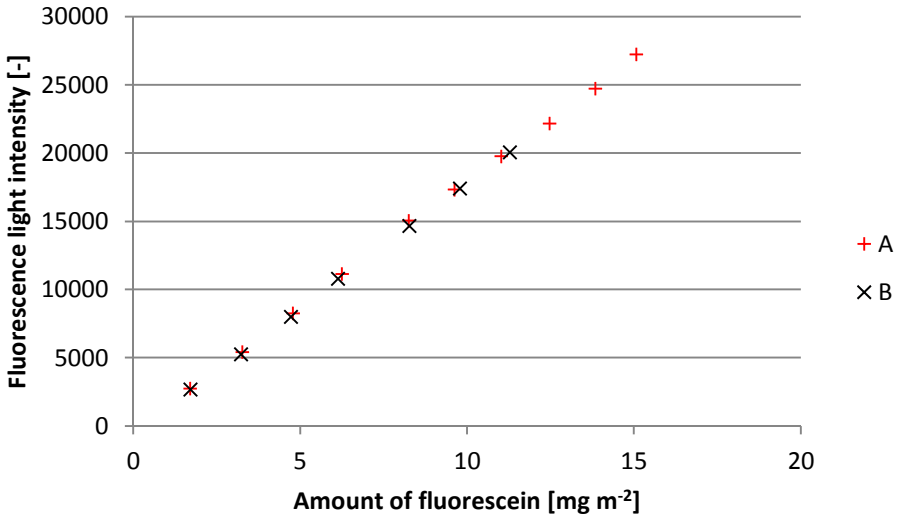

Figure 4. Relationship between amount of fluorescein and fluorescence light intensity (F_{final}) at 2.5 mg^{-1} l^{-1} fluorescein solution.

4. Conclusions and recommendations

According to theoretical background described by Aeby et al (2001) a linear relationship between amount of a fluorescence tracer present and the fluorescence light intensity could be established.

Experimental setup was able to produce detailed images. Additional image processing software was able to perform the necessary corrections and calculations.

Based on this setup and found relationship estimations of puddle size and depth seems possible.

Possible dependency of used fluorescing tracer (fluorescein) on pH should be further investigated.

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