Visible/near infrared spectroscopy for identifying *Salmonella* infected broilers

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Introduction

Bile is produced by the gallbladder and is a fluid with high salt content that aids in the digestion of lipids in the small intestine. Bile is also bactericidal and helps protect the host against common microbes present in food. However, enteric bacteria that are associated with foodborne disease are generally resistant to the action of bile salts. *Salmonella enterica* is especially capable of persistently colonising the gallbladder, which can result in some hosts becoming chronic carriers.[1] When the gallbladder becomes infected, inflammation can result in excretion of large amounts of bile in a manner that changes the color of excreta from brown to green (Figure 2a). Detection of bile discolouration in droppings produced by hens infected with *S. enteritidis* raises the possibility that machine vision techniques might be useful for detection of the pathogen. *S. enteritidis* is the leading cause of foodborne salmonellosis in the world.[2] Testing for the presence of *S. enteritidis* is currently done by collecting and culturing swabs from farms, organs of hens, chick papers, droppings and eggs as described by industry, state and federal regulations or guidelines.[3]

Figure 1. Experimental cages for bile and dropping sample collection.

However, new methods that can help monitor for the presence of *S. enteritidis* on-farm in a manner that saves labour and increases the ability to pinpoint emergence of the pathogen is of interest for controlling foodborne disease associated with *S. enteritidis* in layers. Visible/NIR spectroscopy has the potential to find key wavelengths[4] for bile detection using machine vision technology. This information can be used for further validation with hyperspectral[5] and multispectral[6] imaging for real-time monitoring for healthy broilers. Thus, the objective of this study was to evaluate visible/NIR spectroscopy to identify spectral characteristics of bile in droppings for early detection of broilers infected by *S. enteritidis*.

Materials and Methods

For visible/NIR data acquisition, bile was collected from the gallbladder of *Salmonella*-inoculated birds and droppings were collected from birds in the same cages. Figure 2 shows samples collected for visible/NIR spectroscopic measurements. Each figure presents bile in a glass container (Figure 2a), droppings in the plastic bag (Figure 2b) and droppings with bile mixtures at three different concentration levels (Figure 2c) respectively. Pure bile was inserted in a sample cell with optical quartz surfaces that was specially designed for liquid samples.

Samples

Approximately 5 mL bile was collected from the gallbladder of *Salmonella*-inoculated birds and 200 g droppings were collected from birds in the same cages. Pure bile sample (1.0 mL) was presented in a cylindrical sample cell (38 mm I.D., 0.2 mm depth) with optical quartz surfaces and locking backs. Four droppings inoculated with 0, 0.5, 1.0 and > 1.1 mL bile were presented in cylindrical sample cells (38 mm I.D., 9 mm depth).

Measurements

All samples were scanned 32 times, averaged and transformed to log (1/reflectance) by a scanning monochromator (XDS, Foss NIRSystems, Inc., Laurel, MD) and principal component analysis (PCA) was performed for the identification of bile on the droppings at different concentrations to examine the limit of detection.

Reference paper as:
B. Park, J. Guard, J. Sundaram, W.R. Windham, S. Yoon and K. C. Lawrence (2012).Visible/near infrared spectroscopy for identifying Salmonella infected broilers, in: Proceedings of the15th International Conference on Near Infrared Spectroscopy,
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Results and Discussion

From the preliminary data analysis, it was found that visible/NIR spectroscopy has the potential to determine key wavelengths for bile detection. These wavelengths can be used for further validation with hyperspectral and/or multispectral imaging methods for real-time monitoring of broilers infected by *Salmonella*. From the initial test using droppings (or faeces) only, the wavelengths (absorbance) which represented spectral characteristics of droppings were 448, 978, 1192, 1460, 1780 and 1910 nm respectively (Figure 3a). Peak wavelengths (absorbance) for bile samples were 430, 662, 1460 and 1910 nm respectively (Figure 3b). According to the validation test with mixture of droppings and bile samples at three (0.5 mL, 1.0 mL and >1.1 mL) concentration levels, the key wavelengths (absorbance) of 448, 662, 978, 1192, 1460, 1780 and 1910 nm were identified (Figure 3c). Based on preliminary results, the reflectance wavelength of 544 nm can be used for bile detection on droppings.

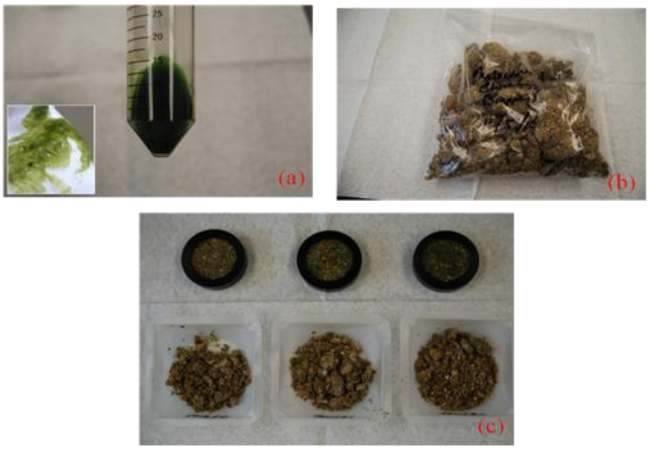

Figure 2. Samples for visible/NIR spectroscopy measurement; (a) bile; (b) droppings; (c) dropping with bile mixtures (0.5, 1.0, > 1.1 mL concentration from left to right.

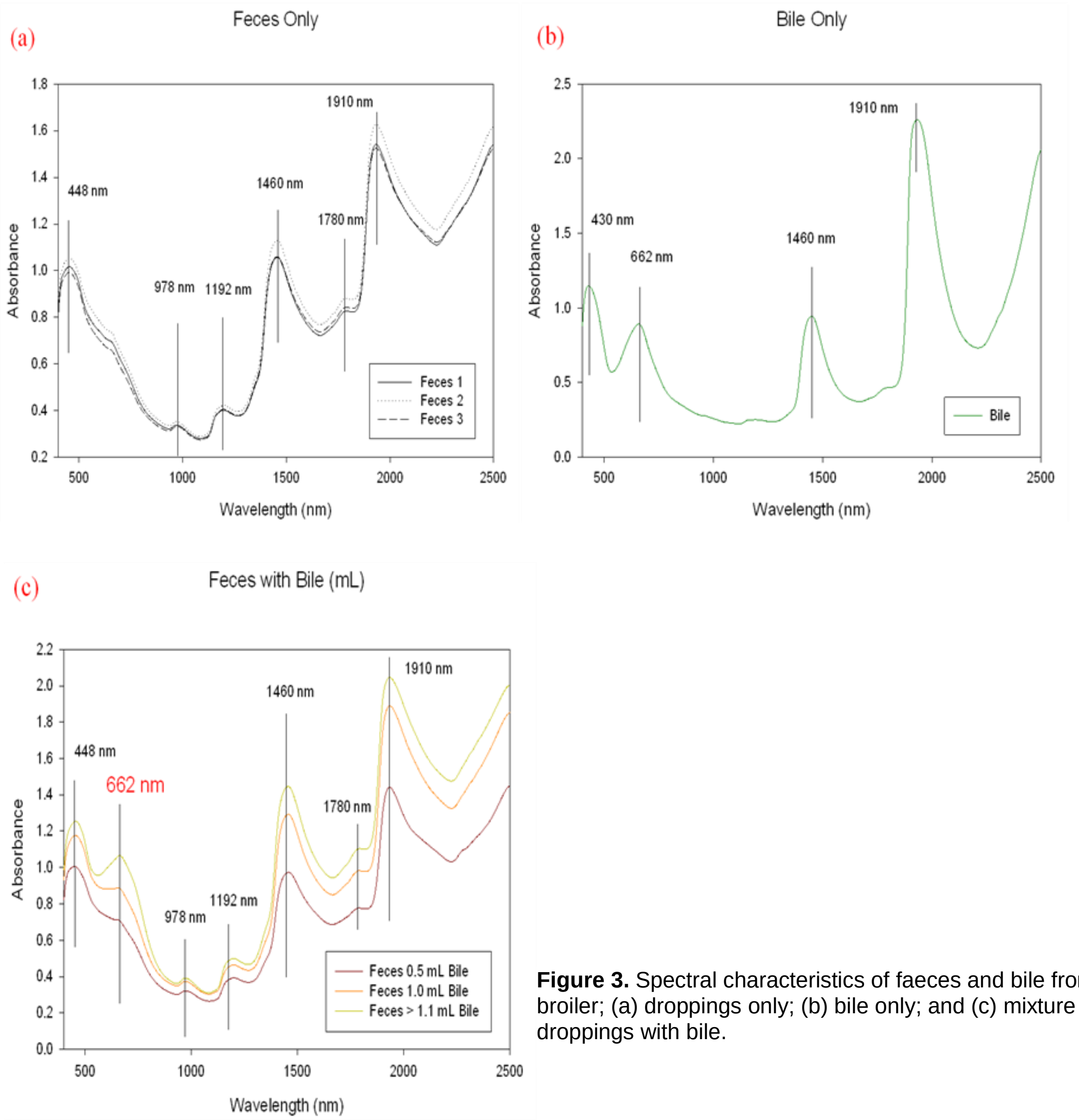

Figure 3. Spectral characteristics of faeces and bile from broiler; (a) droppings only; (b) bile only; and (c) mixture of droppings with bile.

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Principal component analysis (PCA)

Principal component one (PC1) and PC2, which is comprised of 420, 1420 and 1920 nm, accounted for the majority of variation in the samples. From the modelling power (Figure 4) calculated as [1 – square root of (residual variance/variance)], the classification accuracy ranged from 0.84 to 0.99. In the PCA model, wavelengths responsible for identification of bile were 474, 586, 806, 1430, 1880, 2048 and 2370 nm respectively.

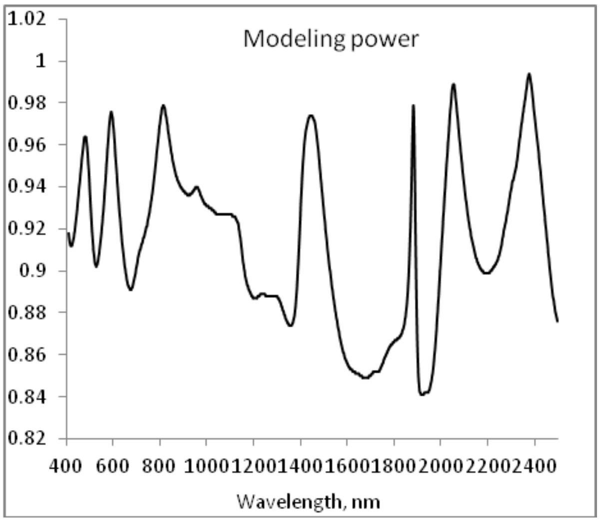

Figure 4. Modeling power with spectral wavelengths.

Conclusion

The potential of visible/NIR spectroscopy to identify bile on poultry droppings was demonstrated. The key wavelengths obtained from this study can be used for further development of hyperspectral and real-time multispectral imaging for monitoring *Salmonella*-infected birds in broiler houses. Although visible/NIR spectroscopy is able to detect various types of faeces obtained from various digestive tracts such as duodenum, cecum and colon, dominant wavelengths identifying droppings mixed with bile were identified in this study. Methods that provide a high degree of automation and consistent surveillance are especially interesting to explore because they can be used in a cost-effective manner to trigger action in the event that signals are detected that raise concern.

References

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