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Fluorescence of Dietary Porphyrins as a Basis for Real-Time Detection of Fecal Contamination on Meat

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Digestion of green plants in the gastrointestinal (GI) tract produces degradation products from chlorophyll that cause ingesta and feces to be highly fluorescent. This property was exploited for development and construction of instruments to noninvasively detect minute quantities of feces on meat samples in real time. The presence of feces on meat products is a primary source of foodborne pathogens, such as *Escherichia coli* O157:H7 and *Salmonella.* This new technology provides a rapid and accurate alternative to the practice of visual inspection and augments more time-consuming biological testing methods. This innovation can assist meat processors and government inspectors in their efforts to provide safe and wholesome food to consumers.

KEYWORDS: Real-time detection; fluorescence spectroscopy; fecal contamination; food safety; chlorophyll degradation

INTRODUCTION

Microbial pathogens in food cause millions of cases of human illness a year according to the Centers for Disease Control and the Council for Agricultural Science and Technology (*1*, *2*). Foods derived from animal products are an important source of human infection and illness. Contamination of red meat and poultry with foodborne pathogens can occur as a result of exposure of the animal carcass to ingesta, feces, or soiled hides during slaughter and processing.

Meat processors, consumers, and government inspectors have all expressed a need for devices that can be used to determine the general level of cleanliness of a carcass following slaughter. Such devices can be useful in determining the presence of fecal or ingesta contamination that has been transferred onto a carcass during the slaughter process. This information can also be important for the improvement of processes and for monitoring the efficacy of preventative and intervention procedures (i.e., carcass washes). Devices capable of automation, high speed, and near real-time analysis would be most useful for industry because they would not interfere with existing slaughter line speeds.

Under current procedures, fecal contamination is detected by unaided visual examination of carcasses and a "zero tolerance" requirement for visible fecal contamination on carcasses is mandated (*3*). However, it is difficult to thoroughly inspect carcasses as they pass by on the rail (up to 400/h in beef plants). It is also difficult to determine if cleansing methods are successfully removing this contamination.

We have previously reported on the use of a fluorescent probe, F420, that is intrinsic to the digestive tract (*4*). This factor is an electron transfer cofactor present in methanogenic bacteria, which are common inhabitants of the digestive tract. Our spectroscopic investigation of F420 revealed an additional fluorescent signal that may be more useful than F420 for feces detection. This signal was detected in the red region of the spectrum and is a consequence of chlorophyll digestion in the gastrointestinal (GI) tract (*5*, *6*). Chlorophyll digestion is a well-known process because its degradation produces metabolites that can result in a pathological condition known as photosensitization. Chlorophyll metabolites that are absorbed from the GI tract will cause photosensitization in animals when liver dysfunction fails to keep these photoreactive compounds out of peripheral circulation (*7*, *8*). When disseminated to tissue exposed to sunlight, such compounds damage adjacent cells through processes of free radical formation causing dermatitis and other characteristic symptoms of photosensitization.

Fluorescence spectroscopy has been commonly used in a variety of biological applications, and its use for detection of contaminants on foods has been previously reported. For example, Alfano (*9*) reported a method for detecting biological molecules and microorganisms by irradiating the sample material with UV light at a wavelength between 250 and 325 nm and measuring the resultant fluorescence. This method was reported to be useful for medical applications as well as for detecting

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spoilage of foods including meats. More recently, Waldroup and Kirby (*10*) and Xiao et al. (*11*) reported methods for detecting contamination (feces and ingesta) on meat or poultry. As described in their patents, meat or poultry is illuminated with UV light and examined for fluorescent emission. Unfortunately, these methods have employed excitation and emission wavelengths that cause high background fluorescence and produce erroneous results. For example, intrinsic fluorescence from aromatic amino acids, such as tryptophan and tyrosine, and from tissue proteins such as collagen contribute to the background fluorescence problem. This intrinsic fluorescence was recognized by Hoerman and Balekjian (*12*), who stated that, "Luminescence of collagen following irradiation with UV light appears to be a universal property of the tissue."

Other work has used electronic imaging devices to detect bioluminescence and fluorescence for specific applications. Bioluminescent bacteria and green fluorescent protein have been successfully used in many areas of biological research. These include in situ *Salmonella* pathogenesis (*13*) and detection of bacteria in water, on vegetables, and on meat (*14*, *15*). Contag et al. (*13*) detected photons transmitted from within the abdomen of mice after oral infection with bioluminescent *Salmonella*. This work used intensified charge-coupled device (CCD) cameras and PC-based imaging software to track the localization of bacteria to specific tissues. Miyamoto et al. (*14*) used a photon-counting camera to rapidly enumerate bacteria in vegetable rinse water via a bioluminescence reaction. Siragussa et al. (*15*) used an intensified CCD camera to study adherence of a bioluminescent strain of *Escherichia coli* O157:H7 to the surface of beef carcasses. Although these experiments used bioluminescence rather than fluorescence as the basis of detection, this work clearly demonstrates the level of instrument sensitivity currently available in the photonics industry.

The technology discussed in this paper describes an improved system for detecting fecal and ingesta contamination on the carcasses of animals using fluorescence spectroscopy. We demonstrate that the chlorophyll metabolites present in feces produce a fluorescent signal that can be distinguished from the background emissions of red meat and poultry and can thus be used for evaluation of carcass cleanliness.

MATERIALS AND METHODS

Animal Care and Sample Collection. All animals were cared for under protocols approved by the Animal Care and Use Committee of the National Animal Disease Center (NADC). Cattle were fed a ration of corn and alfalfa hay unless otherwise noted. Swine were fed a ration consisting primarily of corn, soybean meal, alfalfa meal, and mineral supplement. In some experiments, to define the fluorescent properties of feces, cattle and goats were fed a ration of only water and yellow oat straw for a period of 2−3 weeks. Samples of contents from the rumen and cecum were obtained from surgically fistulated cattle and goats. Feces were obtained directly from the rectum of subject animals. Additional samples of fecal material were also obtained from other animals and other sources as specified.

Steady State Absorption and Emission. Pheophorbide *a* and phylloerythrin dihydrochloride (Frontier Scientific, Inc., Logan, UT) were each used as received (∼95% pure with the major impurity being pyropheophorbide *a*, as analyzed by the supplier). Pyropheophorbide *a* methyl ester (95% pure) was obtained from Sigma-Aldrich (St. Louis, MO). The solvents used were freshly purchased and used as received. Water was of Nanopure quality.

Absorbance spectra were obtained using a Perkin-Elmer Lambda 18 UV−visible spectrophotometer. Excitation and emission spectra were obtained using a SPEX FluoroMax with a 4 nm band-pass resolution. For solid or opaque samples, the front-faced fluorescence technique, with a 45° triangular cuvette, was used.

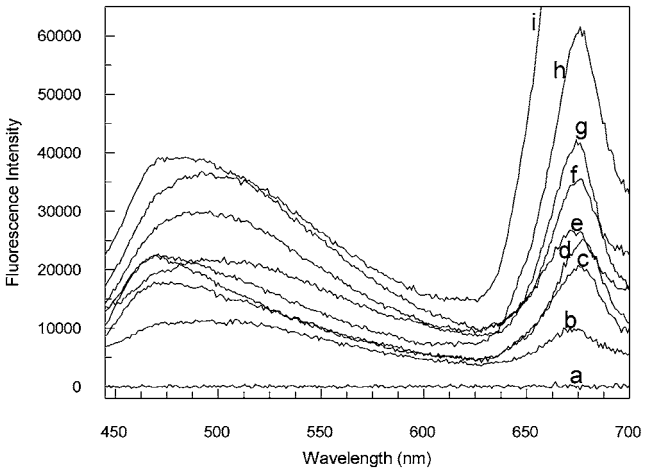

Figure 1. Representative fluorescence spectra of ruminal, cecal, and fecal samples from two different cows and a pig fecal sample. The excitation wavelength was 420 nm. The baseline (a) at zero intensity is the emission from the 50 mM Tris buffer after correction for Raman scattering from water. Each emission spectrum was corrected for Raman scattering by subtracting the contribution of Raman scattering from the Tris buffer. Spectra c, d, and i are ruminal samples from cow 1 and spectrum b is from cow 2. Spectrum h is a cecal sample from cow 2. Spectrum f is a fecal sample from cow 2, spectrum e is a fecal sample from cow 1, and spectrum g is a pig fecal sample. Note the broad band centered at about 490 nm and the intense band centered at ∼670 nm. Both of these bands are signatures of fecal contamination. The prominent emission band near 670 nm is from chlorophyll metabolites.

For the fecal and ingesta samples obtained from animals (cattle and swine) housed at the NADC, emission spectra (**Figure 1**) were obtained by adding small amounts of a fresh sample (50 mg) to 3 mL of 50 mM Tris buffer (pH 7.5). This initial preparation was further diluted (1:50) with buffer and transferred to a 1 cm × 1 cm cuvette. Dilute suspensions were necessary to reduce the opacity of the samples. The excitation wavelength was 420 nm. A reference spectrum of Tris buffer was obtained so that contributions to each sample spectrum from Raman scattering could be subtracted.

Front-faced emission spectra were obtained on clean, freshly cleaved meat samples. Meat samples were subsequently spotted with a few milligrams of fecal material on the front surface (**Figure 2**). The meat samples were placed directly into a 45° cuvette, and the fecal material was spotted onto the meat surface by using a spatula to place the feces between the meat surface and the cuvette face. The amount of fecal material spotted on the meat surface was not quantified since qualitative measurements were desired. The excitation wavelength used to obtain these spectra was 420 nm.

Hand-Held Prototype Detector. The hand-held detector was based upon the design illustrated in **Figure 3**. It was designed to be small (14.6 cm × 31.8 cm × 29.5 cm) and portable. The light source for this working prototype was a CL-1001-Diode Pumped Nd:YVO_4 continuous-wave laser (model No. GCL-050-M, CrystaLaser, Reno, NV) with an output of ∼40 mW at 532 nm. The fiber optic used was a Visionex-Enviva Biomedical Probe: Raman V1.0. This probe consisted of two fiber optic leads, a laser lead and a collection lead. The laser lead carried the laser excitation light through the fiber. It consisted of a single 300 μm silica fiber with a numerical aperture of 0.22. The fiber was fitted with a SMA termination to which a beam collimating lens was attached to aid in coupling the green laser light into the fiber. The collection lead consisted of seven silica fibers each with a 300 μm core. The numerical aperture at instrument end (opposite probe tip) was 0.22 with a SMA termination and collimating lens to collimate the emission light for more efficient transmission to the photomultiplier tubes. The probe tip was a stainless steel needle with a diameter of 1.5 mm.

The green laser light was modulated at a frequency of 220 Hz using a CH-10 Tuning Fork Resonant Chopper (Electrooptical Products Corp., Flushing, NY). The tuning fork chopper was electromechanically driven

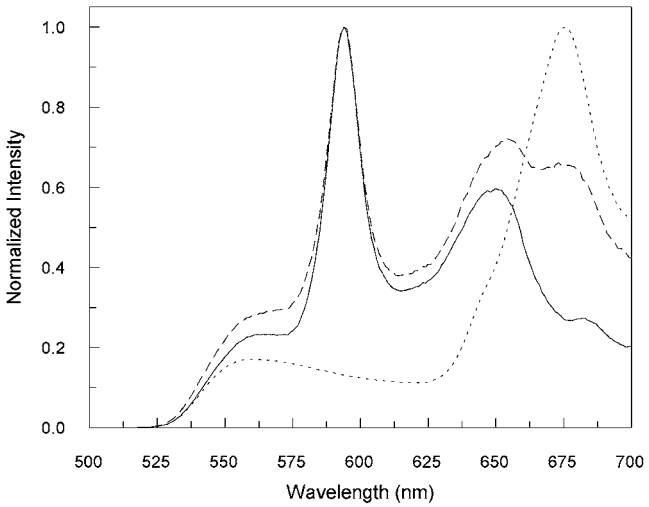

Figure 2. Front-faced fluorescence spectra of beef (—), beef spotted with fecal material (– – –), and feces (- - -). The excitation wavelength is 420 nm. The feces is clearly detected by the emission at 670 nm. The fluorescence at ~600 nm is from myoglobin. The fluorescence minima at ~610 nm and myoglobin fluorescence may be used as an internal standard in normalizing the signal from the contamination. A 555 nm cutoff filter was used to eliminate scattered excitation light.

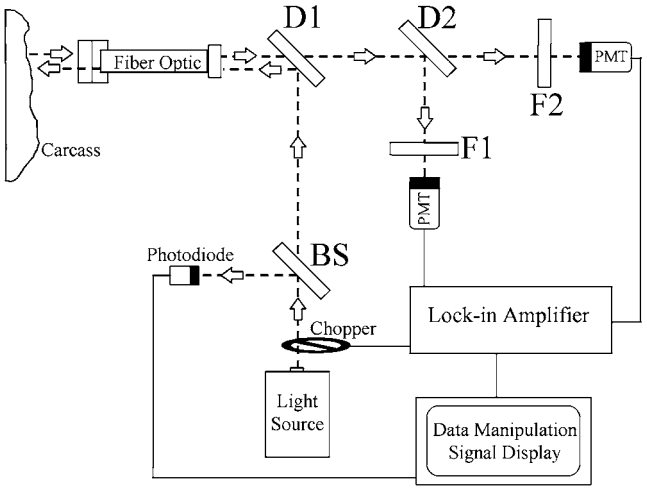

Figure 3. Schematic diagram of an apparatus useful for the detection of fecal contamination. Additional details of the components are given in the text. The beam splitter, BS, was used to divert a small portion of the excitation light for normalization purposes. D1, 532 nm reflecting/610–1037 nm transmitting dichroic mirror; D2, the 610 nm reflecting/670–1030 nm transmitting dichroic mirror; F1, 610 nm interference filter; F2, 670 nm interference filter. The lock-in amplifier employs a 100 ms time constant, which provides for real-time detection.

by a Drive Electronics Type ED Driver, which also provided a square wave reference output signal. The modulation provided by the chopper was sinusoidal with a 50% duty cycle.

The modulated emission from the surface of the sample was collected through the collection fibers and into the lead of the probe. The collection lead consisted of a Thorlabs, Inc., SMA collimating lens to transmit the light more efficiently to a CVI 610 nm reflecting/670−1037 nm transmitting dichroic mirror (D2 in **Figure 3**). Two Hamamatsu H6780-01 Series Photosensor Modules (300−820 nm) were used to detect the light signals. A 610 nm (10 nm band-pass) interference filter (F1) was placed in front of photomultiplier 1 to detect the reflected light from the dichroic, and a CVI 670 nm (10 nm band-pass) interference filter (F2) was placed in front of photomultiplier 2 to detect the transmitted light from the dichroic mirror. Two lock-in amplifiers were employed (Pocket Lock-In Amplifiers, model VK-90, Electro-Solutions, Inc., Flemington, NJ), one for each of the signals detected, 610 and 670 nm. These two signals were subsequently subtracted from each other to provide the corrected signal from the sample. Thus, upon illumination of the sample with excitation light, the intensity of fluorescent light emission was quantitatively measured at wavelengths between 660 and 680 nm (the signal) and between 605 and 615 nm (the reference), and these two values were compared. This yielded an absolute measurement of the fluorescence signal arising from the feces or ingesta.

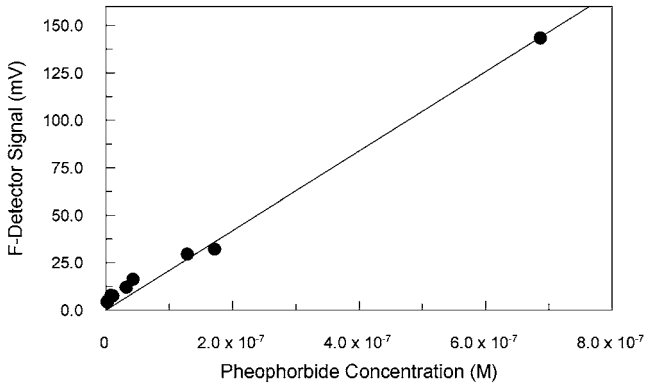

Figure 4. Sensitivity curve for a manufactured device as diagrammed in **Figure 3**. The real-time detection limit based on this plot is 1×10^{-9} M under the respective experimental conditions as outlined within the text. A time constant of 100 ms is employed. The correlation coefficient of the linear fit through zero is 0.987. The slope of the line representing the sensitivity of the device to pheophorbide is 2.00×10^{8} mV/M (200 mV/μM).

A calibration curve illustrating the response and detection limits of the detector to solutions of commercial pheophorbide *a* in ethanol was obtained (**Figure 4**). A 1 mm path length quartz cuvette, which contained the various concentrations of solution, was placed in front of the probe tip. The probe tip was fixed in a horizontal position at a distance of 3 mm from the cuvette surface. The probe tip and cuvette were kept in fixed positions for each voltage reading on the detector.

Imaging Technology. Images of chicken legs, intentionally contaminated with feces, were obtained and analyzed using a Chem Imager 4000 (Alpha Innotech Corp., San Leandro, CA) and the software provided by the manufacturer. The cooled CCD camera was fitted with 610 nm and 670 nm optical filters (10 nm band-pass) for emission images. Chicken samples were illuminated with an excitation light source (actinic blue aquarium light, Energy Savers Unlimited, Inc., Harbor City, CA) fitted with a 430 nm optical filter (10 nm band-pass). Optical filters were obtained from CVI Laser Corp. (Albuquerque, NM).

RESULTS AND DISCUSSION

Fluorescent Marker. The light energy absorbed by chlorophyll within intact chloroplasts is efficiently transferred to other molecules in the photosynthetic pathway. Thus, functional chlorophyll in plants is only weakly fluorescent (*6*). However, during digestion of green plant material in the GI tract, chloroplasts disintegrate and chlorophyll is degraded into various metabolites (*5*). Under these circumstances, chlorophyll and, more importantly, its degradation products dissipate absorbed light energy by mechanisms other than photosynthesis, in particular, fluorescence (*16*). As a consequence of digestion, chlorophyll derivatives cause feces to fluoresce (*17*), and we have taken advantage of this property in our fecal detection system (*18*).

From our experiments, we have concluded that the fluorescent signals shown in **Figures 1** and **2** arise from the degradation of chlorophyll with pheophorbide *a* and pyropheophorbide *a* being the predominant metabolites (**Figure 5**). A comparison of the

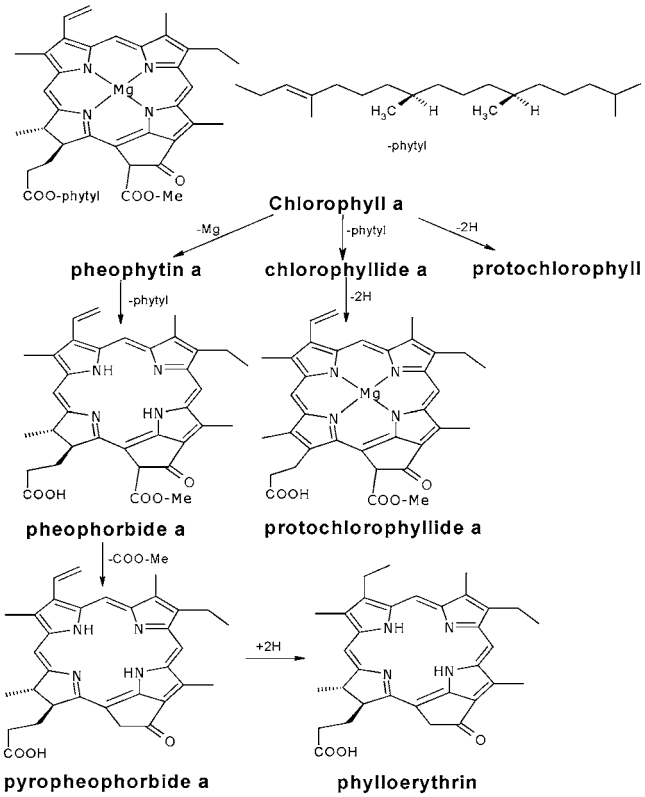

Figure 5. Metabolic pathways for the degradation of chlorophyll (*5*).

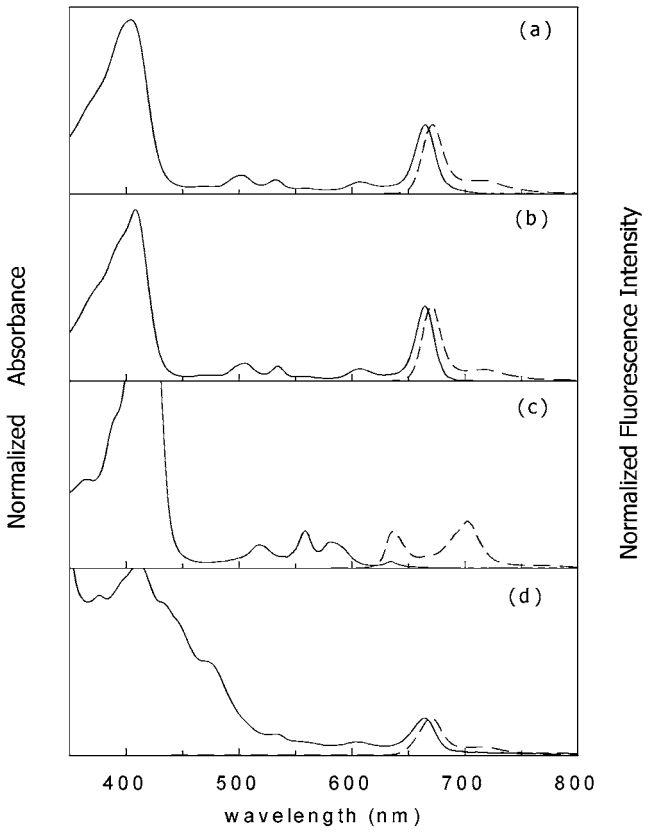

Figure 6. Steady state absorption (solid line) and fluorescence emission (dashed line) of (**a**) pheophorbide *a*; (**b**) pyropheophorbide-*a* methyl ester; (**c**) phylloerythrin dihydrochloride; and (**d**) feces extract in acetone. The excitation wavelength for the emission spectra was 420 nm. The extinction coefficients are (**a**) 120 000 M^{-1} cm^{-1} ± 20% at 404 nm and 47 000 M^{-1} cm^{-1} ± 20% at 665 nm, solvent is acetone; (**b**) 126 000 M^{-1} cm^{-1} ± 7% at 408 nm and 56 000 M^{-1} cm^{-1} m± 6% at 665 nm, solvent is acetone; (**c**) 214 000 M^{-1} cm^{-1} ± 1% at 416 nm, solvent is dimethyl formamide. The feces was extracted in 2-propanol and separated on a silica gel thin-layer chromatography plate using ethyl acetate/heptane (3.5:1) as the mobile phase. Fluorescent spots that had the same R_f as pyropheophorbide-*a* methyl ester were removed, dissolved in acetone, and filtered to obtain the final solution, which was used for the absorption and emission spectra.

spectral and lifetime measurements of the fluorescent compound(s) in feces as compared with those of three commercially obtained chlorophyll metabolites supports this conclusion (**Figures 6** and **7**). Phylloerythrin does not appear to be a major metabolite in feces because its two fluorescent maxima are of nearly equal intensity in the red region and this differs from the spectrum obtained for feces (**Figure 6c**). For the other compounds tested, the second maximum is reduced to a shoulder (**Figure 6a**,**b**). The fluorescence lifetime decays were qualitatively similar for all samples tested. (Other fluorescent compounds in feces have different fluorescence emissions, with maxima between 450 and 550 nm (**Figure 1**).) Therefore, we have concluded that the red emission (650−750 nm) in feces is due to chlorophyll that has undergone digestion in the gut. This conclusion gained additional support from an analysis of the spectra of feces obtained from ruminants fed a diet of yellow oat straw. During consumption of this minimal diet, the GI tract became depleted in its chlorophyll content and the characteristic red fluorescent signal at 674 nm decreased over time. After 2−3 weeks on the straw diet, this red signal was no longer detectable. **Figure 8** presents data for the intensity of the 674 nm band in goats fed on a straw diet over a period of 2 weeks. Similar results were obtained with cattle.

Although diet determines the intensity of the 674 nm signature, normal animal rations do not reduce the signature to a point where it is incapable of being detected with real-time sensitivity. More to the point, we have recently examined the fluorescence spectra of feces from feedlot cattle. Over 100 samples representative of feedlot diets across the United States over a 4 month period beginning in November 2001 were investigated. These feedlot diets were administered to the cattle for a minimum of 80−90 days and contained a variety of feeds, including unusual feed byproducts, such as cotton and sunflower hulls, cookies, tortillas, cereal, and even candy bars. In all cases, the optical excitation and detection criteria described in our paper enabled detection of the feces even when the forage content was quite low, as is the case in several of the feedlot rations.

Fecal Detection. Our detection system is based upon the analysis of specific fluorescent light emitted by an illuminated carcass. Its utility is illustrated in **Figures 1** and **2**, where feces is demonstrated to have a characteristic fluorescence. Dilute solutions of feces or ingesta when irradiated with blue light (420 nm) produced a characteristic spectrum. In addition to a broad emission peak between 450 and 550 nm, the characteristic peak at 674 nm was demonstrated in all of the bovine and porcine samples shown in **Figure 1**. Although these samples emitted light at 674 nm, we have found that there is variability between animals on different diets. These observations support our conclusion that fecal fluorescence is dependent on diet and is due, in large part, to green plant ingestion and chlorophyll metabolism.

Figure 2 demonstrates one method for employing fluorescent (light-emitting) markers in the detection of feces and ingesta on meat. Here, solid cuts of fresh beef were intentionally contaminated with feces. In these experiments, the small amount

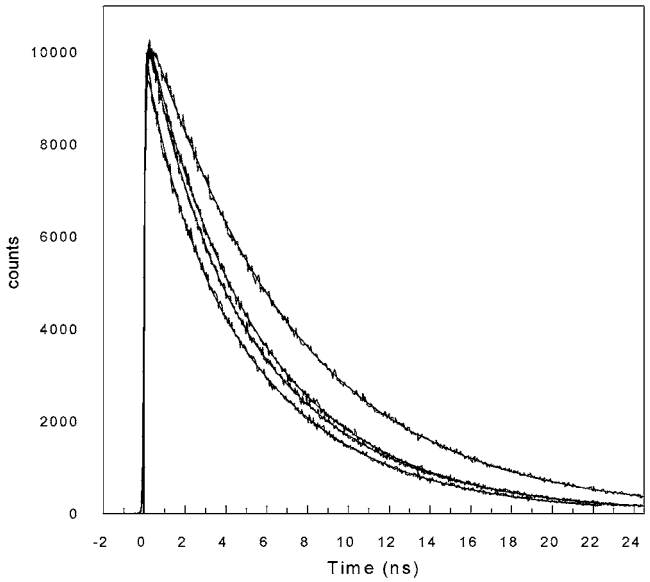

Figure 7. Fluorescence lifetime decays, from right to left: pyropheophorbide-*a* methyl ester, $F(t) = 1.0\exp(-t/7.17\ \text{ns})$; phylloerythrin dihydrochloride, $F(t) = 0.95\exp(-t/6.00\ \text{ns}) + 0.05\exp(-t/1.06\ \text{ns})$; pheophorbide *a*, $F(t) = 0.76\exp(-t/6.08\ \text{ns}) + 0.24\exp(-t/1.81\ \text{ns})$; fecal extract, $F(t) = 0.77\exp(-t/5.74\ \text{ns}) + 0.23\exp(-t/1.22\ \text{ns})$. The samples were excited at 580 nm, and the emission was collected at wavelengths greater than 610 nm using a cutoff filter. $\chi^2 \leq 1.2$ for all data presented. All samples were dissolved in acetone.

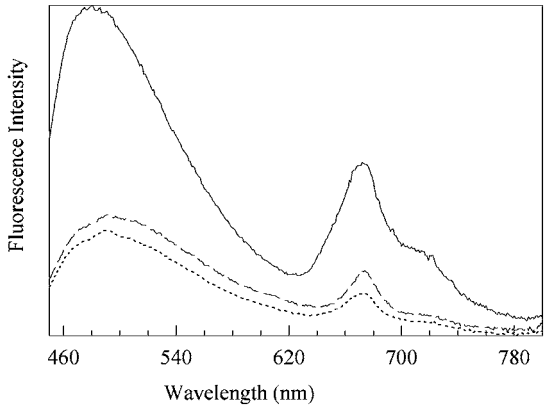

Figure 8. Representative fluorescence spectra of fecal samples from goats on a straw diet. Fifty milligrams of the fecal samples was used to prepare samples suitable for optical measurements, as discussed in the text. The top spectrum (solid) is the signal obtained before the straw diet was initiated; the middle (dashed), 5 days on the straw diet; and the bottom (dotted), 12 days on the straw diet.

of contamination placed on the beef was too small to be seen by the eye. The beef was then placed into a 45° angled glass cuvette, and the sample was excited at 420 nm (4.25 nm band-pass). Fluorescence from the meat sample was detected with a 4.25 nm band-pass, and a colored glass filter was used to discriminate against scattered excitation light. Under these conditions, only contaminated samples showed a characteristic emission at ~674 nm.

To assess the possibility of detecting false positive signals, we present the data in **Figure 9**: fluorescent signals of 1 μM pheophorbide, 1 μM myoglobin, and blood and serum from a Hereford cow eating 90% green alfalfa hay and 10% corn. The pheophorbide signal is overwhelmingly dominant. The maximum of the myoglobin signal is slightly less than 600 nm. Although small amounts of chlorophyll may be in systemic

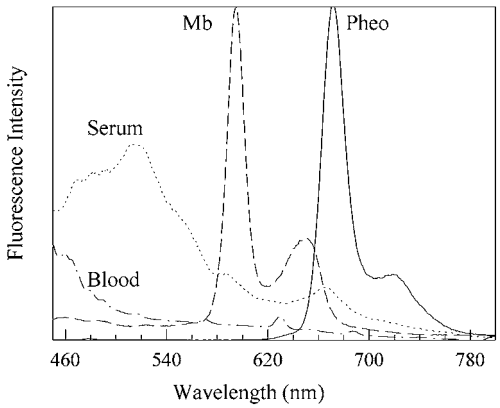

Figure 9. Emission spectra of 1 μM pheophorbide (solid); 1 μM myoglobin (dashed, the intensity has been multiplied by 20 000 in order to normalize it to that of pheophorbide); serum (dotted, the intensity has been multiplied by 60 in order to facilitate comparison); and blood (dash–dot, the emission spectrum was collected front face). Because the experiment for blood was obtained with a front-faced geometry, it is dominated by scattered light, which could not be completely corrected for over the entire spectral region. In all cases, the excitation wavelength was 420 nm.

circulation, it is largely removed by the liver. It is only when the liver is diseased that chlorophyll metabolites escape into peripheral circulation, so it is not surprising that there is no signal at 674 nm in the blood or serum. Consequently, these data confirm our assertion that the spectroscopic signature is not a source of false positives.

Figure 4 is a sensitivity curve for the apparatus illustrated in **Figure 3**. It was obtained in order to estimate the detector response as well as the detection limit of pheophorbide *a*. The real-time detection limit (~3:1 signal-to-noise) based on this plot is 1×10^{-9} M. This corresponds to levels of fluorescent sample that are undetectable by the eye. The calibration curve (**Figure 4**) had a linear correlation coefficient of 0.987 with a slope of 2.00×10^8 mV/M (200 mV/μM). The linear dynamic range of the instrument, according to the calibration curve, spans almost 3 orders of magnitude, 10^{-9}–10^{-7} M. A time constant of 100 ms was used in these measurements, and it provides, essentially, a real-time signal output from the fluorescent marker, pheophorbide *a*. In obtaining this calibration curve, both the 1 mm path length sample cuvette containing the pheophorbide solution and the probe tip remained in fixed positions in order to maintain a constant distance and angle between them. This resulted in rather low noise fluctuations in the signal, ±0.2 mV maximum. These measurements clearly illustrate the sensitivity of the detector to the fluorescent marker.

Figure 10 shows images of a chicken leg that was intentionally contaminated with feces. Fluorescent emission due to fecal contamination was observed at 670 nm (**Figure 10b**) with little contribution from scattered light and background fluorescence. Little of the light emitted at 610 nm was due to the presence of feces (**Figure 10c**). Subtracting the background/scattered light image at 610 nm from the 670 nm image resulted in a corrected image that demonstrates specific fluorescence of feces on the chicken leg surface (**Figure 10d**).

One might ask how much feces this method can detect. It is important to stress that the technology is devised to detect the chlorophyll metabolite marker in feces, which **Figure 4** demonstrates can be accomplished with very high sensitivity. Quantification of feces is, nevertheless, possible but requires a knowledge of the diet, which determines the marker content, and it obliges the quantitative measure of incident light, which stimulates fluorescence, and the fluorescence itself. Such

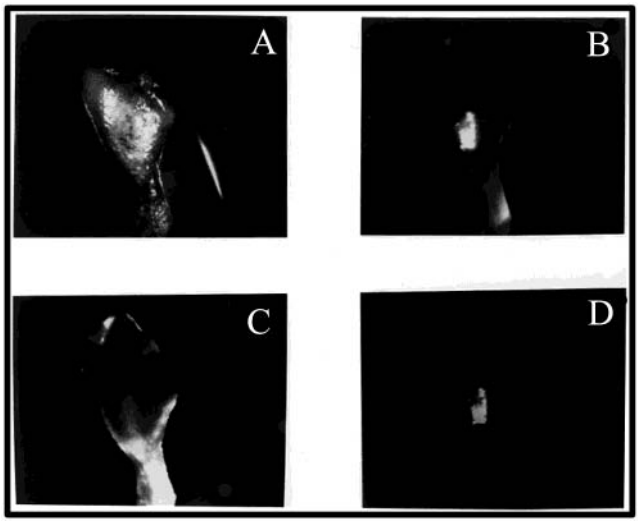

Figure 10. Fluorescent imaging of fecal contamination on a chicken leg. (**A**) Excitation and illumination of the sample with 430 nm light. All emissions were collected using a cooled CCD camera. (**B**) Excitation with 430 nm light. Emission observed at 670 nm. Note the fluorescence from the rectangular smear of feces. (**C**) Excitation with 430 nm light. Emission observed at 610 nm. (**D**) Background-subtracted image, i.e., the signal in panel **C** subtracted from that of panel **B**. This figure provides an illustration of the utility of using the signal at 610 nm as an internal standard or reference in isolating the fluorescent signal from the fecal material.

enhancements of the current method may in time become desirable. However, on a practical level, it should be appreciated that this technology provides a simple and reliable "yes" or "no" answer to the question of whether feces is present or not, which we believe is the most important feature offered by our method, especially as compared with visual inspection, the technique currently employed.

Conclusions. We have demonstrated the utility of our real-time fecal detection method. It is well-suited for detection of the fluorescent markers that arise from chlorophyll digestion. These chlorophyll metabolites are commonly present in the GI tract of herbivorous animals, and these serve as very useful indicators for fecal and ingesta contamination on meat carcasses. As a result, a major source of microbial pathogens such as *E. coli* O157:H7 onto meat can be detected. This apparatus allows for an "instantaneous" detection of contamination by employing a 100 ms time constant. Rapid detection, along with the low detection limit, high sensitivity, and selectivity for the chosen fluorescent markers, provides an extremely useful technology to the meat industry.

In this work, we have identified a specific fluorescent signal in bovine feces that is useful for detecting fecal contamination on animal carcasses. While this methodology appears useful, especially for beef carcasses, this principle of detection may also be useful and appropriate for other foods or objects. Different combinations of excitation and detection wavelengths will extend the usefulness of this technology to other food safety applications and permit its use for other meats, fruits, vegetables, and perhaps for human sanitation and hygiene.

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