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Effect of Spectrally Mixed Hyperspectral Image Pixels on Detection of Cecal Contaminated Broiler Carcasses

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Abstract: Detection of small masses (i.e. 10 mg and less) of fecal contaminants on broiler carcasses presents a significant challenge when using a multispectral imaging system. In contrast to the spectrally noncontiguous multispectral imagery, hyperspectral imagery can be seen as a single image with a contiguous spectrum of reflectance values associated with each image pixel. On a broiler carcass, the spectra may be recognizable as feces provided the contaminant fills or almost fills the pixel in the corresponding scene. Pixels partially filled (i.e. mixed pixels) by a contaminant result in a spectral signature that is a mixture of feces and carcass skin. Mixed pixels with small fecal masses on broiler carcasses can be problematic to accurately detect. The objective of this study was to determine whether hyperspectral imagery offered an improved detection rate of fecal contamination of known mass (2 to 10mg) relative to multispectral imagery; specifically, of fecal matter originating from the cecal. On each of three replicate sample days, twenty-four eviscerated, pre-chilled broiler carcasses were collected from a commercial processing plant. Cecal contents from the same flock were also collected and used to contaminate the carcasses. Carcass halves were first imaged uncontaminated and then imaged again after cecal contents (2, 5, or 10 mg) had been applied to the carcasses. Contaminants were predicted by decision tree (DT) and mixture tuned matched filter (MTMF) classifiers, and results compared. The DT classifier, applied to the multispectral imagery, detected 63, 80, and 100% of the cecal mass applied at about 2, 5 and 10 mg, respectively. The low detection accuracy of the 2 and 5 mg masses was due to some contaminated mixed pixels that either went under-detected or in some cases undetected altogether (false negatives). The MTMF classifier, applied to the hyperspectral imagery, detected 88% of 2 mg and 100% of the 5 and 10 mg contaminants. At an applied mass of about 2, 5, and 10 mg, the MTMF classifier detected 55, 52, and 53%, respectively more cecal contaminated pixels than the DT classifier. The DT classifier incorrectly identified 104, 59, and 56 false positives on carcasses contaminated with about 2, 5, and 10 mg of ceca. On average, these false positives occurred on 36% of the carcasses. The MTMF classifier detected far fewer false positives on 15% of the carcasses.

Key words: Hyperspectral, imaging, poultry, feces, food safety

Introduction

Hyperspectral and multispectral image techniques have been developed and used to detect fecal contaminants on poultry broiler carcasses (Lawrence *et al.*, 2003a; Park *et al.*, 2002; Windham *et al.*, 2003a). The development of these techniques was in response to the Food Safety performance standard (USDA, 1996) which mandates that no carcass can have visible fecal contamination prior to entering the immersion chiller tank. Using a ratio of reflected light at 565nm and 517nm from hyperspectral and/or multispectral images, resulted in high fecal detection accuracies ranging from 92.5-100% (Lawrence *et al.*, 2006; Park *et al.*, 2003; Windham *et al.*, 2003b, 2003c). The fecal detection accuracies reported in the above studies were based on contamination of carcasses with ingesta, duodenum, cecal, and colon material varying in spatial size and location on the carcass. However, Type II errors (false positives) associated with specular reflectance, feathers, edge pixels, cuticle, and scabs were

problematic using detection thresholds of 1.00 and 1.05 (Lawrence *et al.*, 2006; Windham *et al.*, 2005).

Recently, modifications to our hyperspectral imaging system (HIS) have reduced the number of false positives while maintaining fecal detection accuracy (Heitschmidt *et al.*, 2004). The modifications include improved lighting, a new hyperspectral imaging camera, and a decision tree classifier that incorporated an additional third wavelength. The decision tree classifier used a series of binary decisions to separate pixels into classes. Each decision, or node, divided the pixels in an image into two classes based on a user-defined expression. In this case, each node contained a conditional statement designed to determine a given pixel's likelihood of being a contaminant or some other type of feature often spectrally confused with contaminants. In this way, pixels that were problematic for the 565/517-nm ratio could be identified and "redirected" for separate consideration.

More recently, Windham *et al.* (2005) reported the effectiveness of hyperspectral imaging to detect cecal contents (10 to 100mg) applied to broiler carcasses. Detection accuracy was 100% for cecal spots with applied masses of 10, 50, and 100 mg. However, only 50% of the pixels associated with the 10 mg spots were detected. Those contaminant-containing pixels left undetected were a spectral mixture of cecal and uncontaminated skin. Detection of mixed pixels associated with small fecal masses is important in overall contaminant identification because as little as 5 mg of cecal contents can cause a significant increase in the number of *Campylobacter* on eviscerated broiler carcasses (Berrang *et al.*, 2004). The objective of this study was to determine the detection accuracy of the hyperspectral imaging system for cecal contaminants of known mass (2 to 10mg).

Materials and Methods

Samples: Broiler carcasses were collected directly from the shackle line after the inside outside washer in a commercial broiler processing plant as described by Berrang *et al.* (2004). For hyperspectral imaging, twenty-four carcasses were collected on 3 replicate sample trips (n = 72). Concurrent with carcass collection, intestinal tracts were collected by manual removal from the processing line immediately after evisceration. Intestinal tracts were gathered and pooled together in a clean plastic bag.

Carcasses were cut in half with a sanitized bone saw along the dorsal/ventral midline resulting in mirror image halves. Each half was placed into a separate clean plastic bag which was numbered to allow identification of halves from the same carcass. One half of each carcass was randomly selected to be contaminated with cecal contents for imaging and microbiological studies (Berrang *et al.*, 2004) while the other half of the same carcass remained uncontaminated as a negative control.

Hyperspectral imaging system: The hyperspectral imaging system described by Lawrence *et al.* (2003a) and Windham *et al.* (2005) was used to image uncontaminated and feces-contaminated carcass halves. Briefly, the imaging system employed utilized a 12-bit SensiCam QE camera (Cooke Corporation, Auburn Hills, MI) outfitted with a 2/3" format, 1376 x 1040, Peltier-cooled CCD array. The spectrograph was an ImSpector V10E (Specim, Oulu, Finland) with a 30-micron entrance slit and wavelength range of 400 - 1000 nm. Finally, the front lens was a large format C-mount lens (XNP 14/23-0302, Schneider Optics, Hauppage, NY). For illumination of poultry carcasses, six DC-stabilized light sources (MR1600, Gilway Technical Lamp, Woburn, MA) were positioned surrounding the hyperspectral imaging system, providing near direct light

of the carcass. Each light source was outfitted with a 35W MR16 tungsten-halogen lamp (22° beam spread) and a frosted glass diffusion filter. The diffused, near direct illumination provided even illumination across the carcass.

Procedures

Cecal contents and application: In all experiments, ceca were isolated from the intestinal tract and their contents manually pressed out into a clean plastic dish. Cecal contents were pooled and homogenized by vigorous stirring with a sanitized spatula then packed into sterile 5 ml syringes. The syringe was weighed on an analytical scale, cecal contents were applied to one-half of the carcass and the syringe was weighed again. In this way, the mass of cecal contents applied could be calculated. Target masses were 2 mg, 5 mg, and 10 mg. Cecal contents were applied to unbagged carcass halves hanging by the legs in a shackle. After application of the cecal mass and image acquisition, each carcass half was replaced in the same plastic bag. In each replication, 8 carcasses were treated by application of the same approximate mass of cecal contents.

Hyperspectral Imaging: HyperVisual® software (ITD, Stennis Space Center, MS) was used to interface with the imaging system and collect images of the carcasses both clean and after contamination. Camera binning was set at 4 x 2 (vertical x horizontal) and 325 lines were scanned resulting in images with 344 columns, 325 lines, and 520 spectral bands. Image calibration was performed by imaging a 30.5 cm x 30.5 cm 99% Spectralon calibration standard (SRT-99-120, Labsphere, North Sutton, NH), and by collecting accompanying dark current imagery. Once calibration measurements were completed, a carcass half was hung on a standard evisceration shackle at a working distance of 78.7 cm. First, for each sampling date a hyperspectral image was collected of clean uncontaminated carcass halves resulting in seventy-two images. Next, a single known mass of ceca contaminant was applied in a single spot on the upper thigh area of the carcass. After contamination a second hyperspectral image was acquired, resulting in seventy-two additional images.

The clean carcasses and the application of cecal contents were videotaped so that the exact location of the contaminant was documented. While videotaping, a poultry scientist verbally documented any unusual features on the clean carcasses. Some of the items noted on the "clean" carcasses were the locations of feathers, blood clots / hemorrhages, bruises, cuticle, scabs, and numerous other abnormalities.

Image processing: Using the Spectralon and dark current images hyperspectral images were calibrated to

percent reflectance (Lawrence *et al.*, 2003b) and spectrally smoothed by boxcar averaging over a 19-nm bandwidth using HyperVisual. Images were then spectrally re-sampled to mimic the bandpass filters (517±5nm, 565±5nm, and 802±10nm) which have been previously used to detect fecal contamination (Park *et al.*, 2003; Lawrence *et al.*, 2003a; Windham *et al.*, 2005). These spectrally re-sampled images constituted the multispectral imagery for this study. From these multispectral images, two sets of image mosaics were then created: one containing the clean bird images and one containing the contaminated bird images. This was done for each of the three sampling dates, resulting in six image mosaics. The mosaics enabled simultaneous processing and offered a synoptic view of processing results. ENVI software (ITT Visual Information Solutions, Boulder, CO) was used for image processing and analysis.

A decision tree (DT) classifier (Heitschmidt *et al.*, 2004) was applied to each mosaic, producing a Boolean output image with contaminates identified and set equal to one. The classifier delineated contaminates based on the ratio of reflectance values at 565 and 517nm. In addition, a third term (802nm) was used to reduce false positives.

In an effort to improve the detection of the spectrally mixed pixels, results of the decision tree classification were compared to a more robust hyperspectral classification technique, referred to as ENVI's "hourglass" processing flow. The ENVI hourglass processing technique takes hyperspectral data sets that are highly correlated in nature, reduces their dimensionality, determines which pixels are spectrally pure (called spectral end members), and finally classifies the imagery based on the selected spectral end members. Spectral end members in this case were primarily ceca contaminants. The spectral classifier chosen for this study was the Mixture Tuned Matched Filter (MTMF).

For the MTMF classifier, a series of new mosaics were created based on contaminant mass (2 mg, 5 mg, and 10 mg) and contained 353 spectral bands ranging from 450 nm - 900 nm. For each mosaic, dimensionality reduction began with the application of ENVI's two-step principal component analysis (PCA) technique known as a Minimum Noise Fraction (MNF) rotation. A mask was used when calculating MNF statistics that prevented extraneous background feature space from influencing the PCA rotations. Using only those MNF-transformed image bands with eigenvalues = unity, a Pixel Purity Index (PPI) procedure was run to determine which pixels were spectrally unique. Those pixels found to be spectrally unique were loaded into ENVI's n-Dimensional Visualizer where spectral end members were chosen based on their location relative to the represented data cloud. Using this tool, spectrally "pure"

pixels were found located outside of the data cloud. For each mosaic the pixel with the highest PPI score that was associated with cecal contamination was selected as training data for a MTMF supervised classifier, thus each mosaic had one spectral signature used as training data for the classifier. The MTMF classifier performed a matched filtering classification based on the input training data and produced an output image containing two bands: a matched filter band and an in feasibility band. The latter band was a measure of noise expressed in sigma units. Contaminates in the original images were identified by loading these two image bands into a 2-D scatter plot and lassoing pixels with a high matched filter score and low in feasibility score.

Using ENVI's region of interest tool (Windham *et al.*, 2005) the number of pixels detected as cecal contaminates was counted for both the DT and MTMF classifiers. Results from both classifiers were compared for the number of contaminant pixels detected (i.e. true positives) as well as false negatives and false positives. Determination of true and false positives was aided by referencing the video associated with each image.

Results and Discussion

Table 1 shows the detection accuracy of the DT classifier for broiler carcasses contaminated with about 2 to 10 mg of ceca material. The DT classifier with a threshold of 1.05, detected 63, 80, and 100% of the cecal mass applied at 2, 5, and 10 mg, respectively. The low detection accuracy of the 2 and 5 mg masses was due to contaminated pixels that either went under-detected or in some cases undetected altogether (false negatives). There will always be a trade-off between detection accuracy due to algorithm detection thresholds and the occurrence of false positives. Small (= 10 mg) contaminant masses are difficult to detect, but can be determined by decreasing the algorithm detection threshold. Windham *et al.* (2005) reported 100% detection of cecal mass (averaged over 10, 50 and 100 mg) using a threshold of 1.00 and 1.05. However, at the 1.00 threshold, 252 false positives were detected on 84% of the carcasses. The 2 and 5 mg cecal masses were small in spot size and the low concentration of spectrally pure cecal pixels made detection difficult at a 1.05 threshold. Typically, for contaminate masses less than 10 mg there were a number of spectrally mixed pixels around the perimeter of the contaminant that went undetected. These pixels were a spectral mix of cecal content and skin as indicated by the reflectance peaks for myoglobin in the skin (Windham *et al.*, 2005). For contaminants greater than 10 mg, mixed pixels still occurred at the boundary between the contaminant and skin, but their detection was not critical because a greater proportion of the pixels were pure contaminants and thus easily detected.

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Table 1: Detection accuracy for the decision tree classifier of broiler carcasses contaminated with cecal contents

Cecal mass (mg)	Contaminated pixels (n)	Detection accuracy (%)	False negatives (n)
2.3 ± 0.5	48	63	9
5.5 ± 0.9	119	80	5
10.1 ± 1.2	175	100	0

Table 2: Detection accuracy for the mixture tuned matched filter classifier of broiler carcasses contaminated with cecal contents

Cecal mass (mg)	Contaminated pixels (n)	Detection accuracy (%)	False negatives (n)
2.3 ± 0.5	106	88	3
5.5 ± 0.9	249	100	0
10.1 ± 1.2	370	100	0

Table 3: False positives identified by DTC [a] and MTMF [b] classifiers on boiler carcasses contaminated with cecal contents

	DTC		MTMF	
Cecal mass	False positive pixels (n)	False positive carcasses (%)	False positive pixels (n)	False positive carcasses (%)
2.3 ± 0.5	104	33	43	12
5.5 ± 0.9	59	38	4	17
10.1 ± 1.2	56	38	12	17

[a]Decision tree classifier. [b]Mixture tuned matched filter classifier

As compared with the DT classifier, the MTMF classifier offered a significant improvement. The MTMF classifier detected 88% of 2 mg and 100% of the 5 and 10 mg contaminants (Table 2). At an average applied mass of 2.3, 5.5, and 10.2 mg, the classifier detected 55, 52, and 53%, respectively more cecal contaminated pixels than the DT classifier.

The original objective for the development of the DT classifier and the addition of a third wavelength (802 nm) was to reduce the number of false positives (detected as contaminant but no contaminant evident) identified on carcasses with the ratio of reflectance images at 565 and 517-nm. Heitschmidt *et al.*, (2004) reported the classifier correctly identified 99% of 1030 contaminants applied to 56 carcasses with only 25 false positives. False positives identified by the DT and MTMF classifiers are shown in Table 3. The DT classifier incorrectly identified 104, 59, and 56 false positives on carcasses contaminated with 2.3, 5.5, and 10.1 mg of cecal contents (Table 3). On average, these false positives occurred on 36% of the carcasses. Windham *et al.*, (2005) reported similar results using the 565/517-nm image ratio, where 65 false positive were incorrectly identified on carcasses contaminated with 10, 50, and 100 mg of cecal contents. These false positives occurred on 22% of the carcasses. By contrast, the MTMF classifier detected far fewer false positives occurring on only 15% of the carcasses.

Samples from this study were used by Berrang *et al.* (2004) to determine the effect of cecal contents (2 to 100 mg) applied to broiler carcasses on bacterial counts. Numbers of *Campylobacter, Escherichia coli,* other coliform species, and total aerobic bacteria were obtained on carcass halves with and without the addition of cecal contents. When about 10 mg of cecal contents was applied to carcasses, a significant increase in all bacterial populations monitored, except total aerobic bacteria, was found. When about 5 mg was applied a significant increase was observed for *Campylobacter* counts. However, the numbers of *E. coli*, coliforms and total aerobic bacteria did not increase. The increase in *Campylobacter* numbers was fairy small, less then 1 log level. When only about 2 mg of cecal contents was applied, no significant increase on any of the bacteria enumerated was noted.

While the hyperspectral imaging system and various fecal detection algorithms can detect small fecal contaminants, the occurrence of false positives has been problematic. The average number of ground truth positive pixels for an applied mass of about 2 mg was 5 ± 1.7. Many of the false positives in this study were of the single-pixel-per-bird variety as well as aggregates of 3 to 5 pixels. Since contaminants of this size do not increase bacterial counts, one could reason that false positive pixels ranging from 1 to 5 pixels can be ignored and filtered out. Elimination of these false positive pixels from the DT and MTMF classifier results reduces the false positive carcasses to 19 and 4%, respectively.

Conclusions: A decision tree (DT) and mixture tuned matched filer (MTMF) classifiers were used with a hyperspectral imaging system to detect cecal contamination of broiler carcasses. The DT classifier, applied to the mulitspectral imagery, detected 63, 80, and 100% of the cecal masses applied at about 2, 5, and 10 mg. The low detection accuracy of the 2 and 5 mg masses was due to some contaminated pixels that either went under-detected or in some cases undetected altogether (i.e. false negatives). These pixels were partially filled (i.e. mixed pixels) by the contaminant and resulted in a spectral signature that was a mixture of feces and carcass skin. Mixed pixels containing only a small amount of fecal mass were difficult to accurately

detect. In addition, the DT classifier incorrectly identified false positives on 36% of the carcasses. The MTMF classifier had higher detection accuracy compared to the DT classifier, but incorrectly identified false positives on 15% of the carcasses. In the case of both classifiers, a significant number of the false positives were of the single-pixel-per-bird variety or aggregates of 3 to 5 pixels. Contaminants of this size do not increase bacterial counts of broiler carcasses. Elimination of these pixels from the DT and MTMF classifier results reduce the false positive carcasses to 19 and 4%, respectively.

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