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Detection of Ingesta on Pre-Chilled Broiler Carcasses by Hyperspectral Imaging

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Abstract: The contents of the upper digestive tract (i.e. crop, proventriculus and gizzard) may serve as a source of carcass contamination during broiler processing. The crop as been identified as a source of *Salmonella* and *Campylobacter* on contaminated carcasses and is more likely to rupture than the ceca during commercial evisceration. The objective of this study was to determine the effectiveness of hyperspectral imaging for detecting ingesta contamination spots varying in mass from the crop and gizzard. Pre-chilled broiler carcasses were collected from a commercial processing plant. Crop and gizzard contents were also aseptically collected and enumerated for *Campylobacter,* coliforms, *E. coli* and total aerobic bacteria. Broiler carcasses were imaged and then contaminated with a spot of known mass (10, 50, or 100 mg) of crop or gizzard contents. Carcasses were then re-imaged. The imaging system correctly detected 100% of the crop and gizzard contents regardless of the mass or spot size. However, not every pixel associated with a given spot (contaminant ground truth) was detected. Detection of crop and gizzard content contaminant ground truth pixels averaged 72 and 53%, respectively. The mean number of bacteria in the crop contents were as follows: *E. coli* 4.0 log, coliforms 4.1 log, and total aerobic bacteria 5.7 log CFU/g of crop contents. Crop contents in the current study were *Campylobacter* negative. Applying crop contents in the amounts of about 9, 54, and 231 mg resulted in significant ($P<0.05$) increases in all bacterial population measured, with the biggest increase being noted for total aerobic bacteria. Gizzard contents contained only 4.6 log CFU/g of total aerobic bacteria. The total added bacterial load from contamination with known amounts of crop and gizzard contents did not significantly increase whole carcass counts of all bacteria enumerated. Based on these counts and numbers of bacteria found in gizzard, carcass contamination with visible ingesta does not appear to significantly increase bacterial load.

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

Introduction

The contents of the upper digestive tract (i.e., crop, proventriculus, and gizzard) or ingesta may serve as a source of carcass contamination during processing. *Salmonella*, *Campylobacter*, and *E. coli* have been found in the contents of the upper digestive tract. Hargis *et al.* (1995) reported that 286 of 550 crops from a commercial broiler processing plant were *Salmonella*-positive. Byrd *et al.* (1998) isolated *Campylobacter* from crop contents in 7 of 9 commercial broiler flocks. Musgrove *et al.* (2001) reported broiler crops collected in a commercial slaughter plant were positive for *Campylobacter* in 95 to 99% of samples, with average counts of 3.6 log CFU. *E. coli* has been recovered at levels of 4.4 log CFU from broiler crops (Berrang *et al.*, 2000).

Higher populations of pathogenic bacteria are found in the cecum, cloaca, ileum, and than in the crop (Barrow *et al.*, 1988). Although levels tend to be lower in the crop compared to other sections of the gastrointestinal tract, the crop has been observed to be a source of carcass contamination during processing, especially when accidentally ruptured during evisceration (Hargis *et al.*, 1995 and Ramirez *et al.*, 1997). In the processing plant fecal and ingesta contaminants are a potential food safety risk. The U.S. Food Safety Inspection Service regulations require that no poultry carcass can have visible fecal contamination prior to entering the ice-water chiller tank (USDA, 1996).

In an effort to provide poultry processing facilities with an accurate and objective means to inspect poultry carcasses, the Poultry Processing Research Unit at the USDA's Agricultural Research Service has developed hyperspectral and multispectral imaging techniques to detect fecal contaminants on poultry carcasses. Previous research using hyperspectral imaging has shown fecal detection accuracies ranging from 92.5 to 100% (Park *et al.*, 2003; Lawrence *et al.*, 2003; Liu *et al.*, (2003). Recently, Windham *et al.* (2005) reported the effectiveness of hyperspectral imaging to detect and estimate carcass microbiology from cecal contamination of known mass on pre-chilled broiler carcasses. The

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imaging system correctly detected 100% of the cecal spots applied at 10, 50 or 100 mg. Berrang *et al.* (2004) using the same samples reported that carcass halves with 5 mg or more of cecal contamination had higher numbers of *Campylobacter* than those without ($P< 0.01$). Therefore, it is important to keep intestinal content contamination to a minimum during processing. The objective of this study was to determine the effectiveness of hyperspectral imaging system to detect ingesta contamination of known mass from both crop and gizzard and estimate the effect of ingesta contamination on pre-chill broiler carcass microbiology.

Materials and Methods

Samples: Twenty-four broiler carcasses for imaging 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). Concurrent with image carcass collection, 10 additional uneviscerated broiler carcasses were collected at the shackle transfer point just prior to evisceration and placed individually placed into plastic bags. Neck skin was aseptically cut and pulled away from the uneviscerated carcasses to manually isolate the crop. A solution of 70% ethanol was applied to the crop surface and sterile scissors were used to cut a slit (approximately 1 cm) in the crop. Sterile PBS (2ml) was pipetted into the crop which was then manually massaged for 1 min and all recoverable liquid and contents were removed from the crop.

The visceral cavity of each carcass was opened to expose the gizzard. Ethanol solution (70%) was applied to the gizzard surface and sterile scissors were used to cut open the gizzard. Contents were scraped into a sterile Whirlpak bag with a sterile spatula.

Crop and gizzard contents were cultured and enumerated for total aerobic bacterial, *Campylobacter*, coliforms and *E. coli* as described by Berrang *et al.* (2004).

All bacterial count data were transformed to $\log_{10}$ CFU/g for statistical analysis. Difference in numbers due to contamination mass were tested by analysis of variance using the General Linear Models procedure of SAS (SAS, 2000), and significant differences reported at the $P<0.05$ level.

Hyperspectral Imaging System: The hyperspectral imaging system described by Heitschmidt *et al.* (2004) was used to collect frontal view images of uncontaminated carcasses and carcasses contaminated with crop and gizzard contents. Briefly, the system consisted of a 12-bit camera with a Peltier-cooled CCD (SensiCam QE, Cooke Corporation, Auburn Hills, MI), a spectrograph (ImSpector V10E, Specim, Oulu, Finland), focal plane scanner (ITD, Stennis Space Center, MS), and front lens (XNP 14/23-0302, Schneider Optics, Hauppage, NY). Also included with the imaging system were six tungsten halogen lamps (Gilway Technical Lamp, Woburn, MA), a personal computer and HyperVisual® software (ITD). The camera, spectrograph, and software were all upgrades relative to previous studies (Lawrence *et al.*, 2003). A color digital camcorder was also used to document the application, type, and location of each contaminant.

The six light sources were positioned surrounding the hyperspectral imaging system. Each light source was outfitted with 35W MR16 tungsten halogen lamps (22° beam spread) and frosted glass diffusion filters. Each light source was positioned and aimed to ensure maximum illumination of the vent and wing cavities and to minimize shadowing.

Procedures

Crop and Gizzard Contents and Application: Pooled crop or gizzard contents were applied on the carcass with a spatula. The spatula with crop or gizzard contents was weighed on an analytical scale, contents were applied to the carcass breast and the spatula was weighed again. In this way, the mass of content applied could be calculated. Target mass was 10, 50 and 100 mg. Crop and gizzard contents were applied to carcasses hanging by the legs in a shackle. Eight carcasses were treated by application of the same approximate mass of gizzard contents on the left breast and crop contents on the right breast.

Hyperspectral Imaging: At the beginning of each day's imaging, HyperVisual software was used to collect a series of images for calibration, including system noise (ie. dark current), 99 % spectralon reflectance panel, and spectralon gradient panel. These images were used to calibrate subsequent carcass images to percent reflectance and to validate the calibration. Once calibration measurements were completed, a carcass was hung on a standard evisceration shackle, which was welded to a stainless steel support rod, and imaged immediately. Black cloth was hung behind the carcass to provide contrast between the carcas and background. HyperVisual software was used to control the camera, which was set at 4 by 2 binning resulting in 320 horizontal spatial pixels and 512 vertical spectral pixels measured per line-scan image. The exposure time was 50 ms. and it took about 40 s. to collect a 400 line-scan image (vertical spatial) needed to image an entire carcass. After an uncontaminated ("clean") carcass was imaged, a single target mass of gizzard contents was applied as one contaminant spot to left side of the breast and a single target mass of crop contents to the right side of the breast.

Image processing: Once a hypercube was created, the data were calibrated to percent reflectance values as

described earlier (Lawrence *et al.*, 2003a). The data were spectrally smoothed by boxcar averaging over a 19-nm bandwidth using HyperVisual. Images were then spectrally resampled to mimic the bandpass filters (517nm ±5 nm, 565 ±5 nm, and 802 ±10 nm) used in a three-band common aperture camera (MS3100, Redlake, San Diego, CA) that is being prepared for eventual on-line inspection. Two sets of image mosaics were then created: one containing the clean carcass images and one containing the ingesta contaminated carcass images. The mosaics enabled simultaneous processing and offered a synoptic view of processing results. ENVI software (Research Systems, Inc. Boulder, CO) was used for image processing and analysis.

A decision tree classifier (Heitschmidt *et al.*, 2004) was applied to each mosaic, producing a Boolean output image with gizzard and crop contaminates identified. Briefly, the decision tree classifier used a series of binary decisions to separate pixels into classes. Each decision 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. In addition, the third term (802 nm) was used to further reduce false positives.

Results and Discussion

Food safety performance standard (USDA, 1996) regulations mandate that no fecal spots of any size can appear on carcasses prior to the chiller. Therefore, it is important to determine the lowest possible fecal spot size or mass that can be accurately detected by hyperspectral imaging system (HIS) which is of microbiological significance. Windham *et al.* (2005) reported that a 10 mg mass of cecal contaminant could be accurately detected by HIS and this amount of *Campylobacteria*-positive cecal contents added a significant number of cells to a broiler carcass (Berrang *et al.*, 2004). Small (< 10 mg) contaminants are difficult to detect, but can be determined by decreasing the algorithm detection threshold. However, with low (< 1.02) detection thresholds, Type II errors (false positives) increase significantly.

Recently, modifications to our 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 algorithm with an additional wavelength. Additional lighting, targeted at critical areas of the carcass, has resulted in fewer shadows and less glare. New components in the hyperspectral imaging camera have effectively removed misregistration inherent in earlier models and has simplified wavelength and reflectance calibration. The addition of a third wavelength (802 nm), coupled with a decision tree processing approach, has significantly reduced false positives associated with the 565/517-nm ratio.

For each carcass image, regions of interest (ROI's) were selected (Windham *et al.*, 2003) of the ingesta mass applied to the carcass and used to determine the number of true positive pixels of the contaminant mass (ie. contaminant ground truth pixels). As such, the contaminant ground truth pixels could be used to determine the percentage of ingesta contaminant pixels detected Windham *et al.* (2005). Although the HIS detected 100% of the gizzard and crop contaminants, not every pixel associated with a given spot (as determined by the ground truth ROI's) was detected. The contaminant ground truth pixels and contaminant pixel detection accuracies are shown in Table 1. The contamination mass was similar for gizzard and crop and close to the target mass, except for the highest crop content mass. Inconsistencies in crop content mass were the result of a high proportion of water and undigested feed. As such it was difficult to weigh and apply to the carcass. The number of ground truth pixels associated with gizzard and crop content contaminants were similar for both 10 and 50 mg target mass. Ground truth pixel counts for 100 mg crop content contaminants were significantly larger with the highest occurring at an applied mass of 231 mg. Gizzard and crop content ground truth pixels were smaller than those associated with cecal content ground truth pixels in a previous study (Windham *et al.*, 2005). The number of cecal content ground truth pixels averaged 59, 170, and 272 for a target mass 10, 50, and 100 mg, respectively, as compared to 11, 75, and 125 for ingesta. Again, it was the high water concentration and feed particles of the crop contents that resulted in a smaller contaminant "spot" per mass.

Detection of the gizzard content contaminant ground truth pixels averaged 53%, regardless of mass (Table 1). The detection of crop content contaminant ground truth pixels was less consistent, with pixel detection rates of 60, 91, and 65% for a mass of 9, 54, and 231 mg, respectively. Fig. 1 shows images of carcasses contaminated with gizzard and crop contents and pixels detected. In all images, gizzard contamination is on the left side of the breast and crop contents on the right. The HIS correctly detected 66, 78, 76, 42, 86, and 55% of the pixels known to be contaminated with gizzard and crop contents, respectively. Fig. 1, plate e and f shows a typical scenario for crop contents applied at an average of 231 mg. At that mass, the contaminant would run down the carcass, dispersing into multiple spots. Individual spots generally contained only a few pixels which were difficult to detect.

Table 1: Contaminant ground truth detection accuracy and 95% confidence intervals from broiler carcasses contaminated with gizzard and crop contents

Gizzard Contents			Crop Contents		
Contaminant mass (mg)	Ground truth (n)	Accuracy (%)	Contaminant mass (mg)	Ground truth (n)	Accuracy (%)
9.3±4.5	12±4	53.3±18.7	8.8±4.1	10±2	60.0±14.7
61.0±5.1	79±9	53.2±9.6	53.6±7.4	70±6	91.4±4.0
120.0±10.5	107±8	53.3±13.5	231.4±59.8	142±22	64.8±13.1

Table 2: Mean bacterial counts (log CFU/crop content mass) and 95% confidence interval of crop content contamination mass

Crop content mass	*Campylobacter*	*Escherichia coli*	Coliform	Total aerobic bacteria
8.8 ± 4.1	ND[a]	1.86[b]±0.2	1.96[b]±0.2	3.56[b]±0.2
53.6 ± 7.4	ND[a]	2.72[c]±0.1	2.82[c]±0.2	4.42[c]±0.1
231.4 ± 59.8	ND[a]	3.29[d]±0.2	3.42[d]±0.1	5.09[d]±0.1

[a]ND, none detected. [b,c,d] Means in columns lacking a common superscript differ ($p < 0.05$)

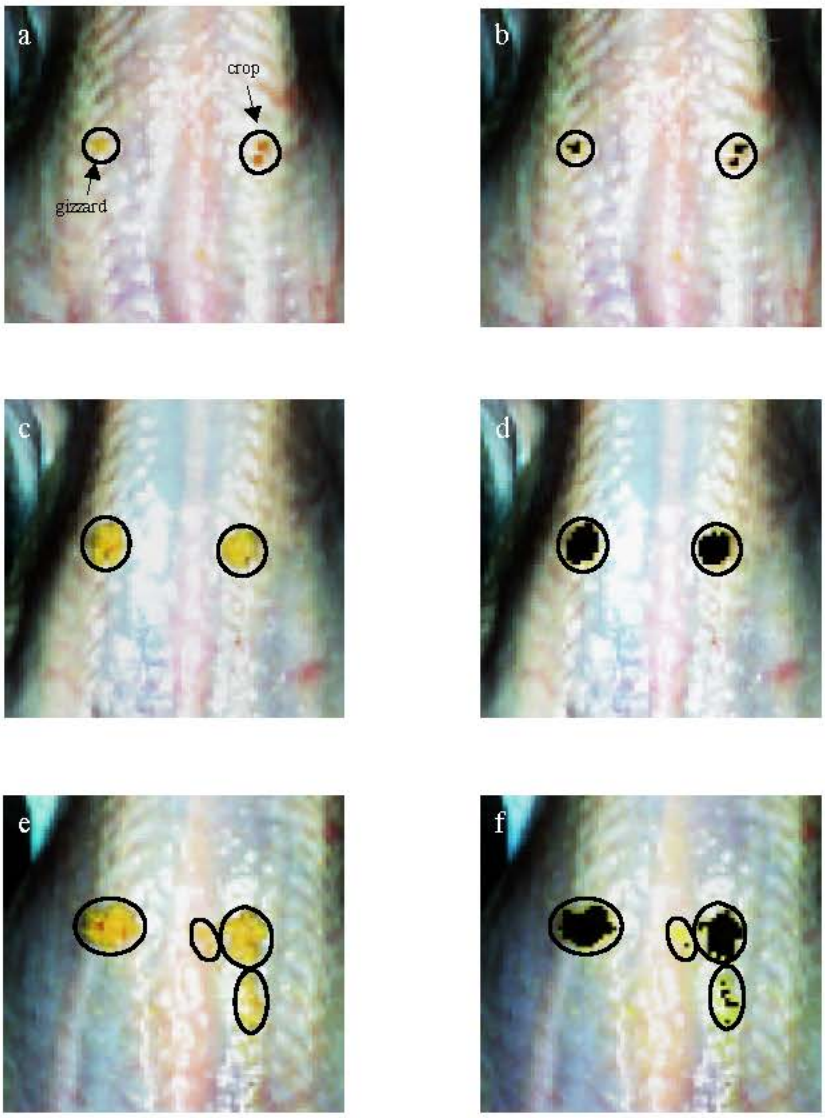

Fig. 1: Composite image of a 10, 50, and 200 mg (Fig. 1 a, c, and e) target mass of gizzard and crop contents and contaminated pixels detected (Fig. 1. b, d, and f).

Higher ground truth pixel accuracies were found in the present study than reported by Windham *et al.* (2005) for cecal content contamination. Averaged across cecal mass, the detection accuracy was 74, 55, and 35% using a contaminant threshold of 1.00, 1.05, and 1.10, respectively. In the current study, averaged across ingesta mass, the accuracy was 53 and 72% using a contaminant threshold 1.10. In addition, Windham *et al.* (2005) report 266 false-positives contaminants (detected as contaminant but no contaminant present) were incorrectly identified, where as in the present study only 28 false-positives were found. Higher detection accuracy and less false-positives was due in part to a combination of hardware and software changes mentioned earlier that have improved the overall performance of contaminant detection based on data collected with the HIS.

Microbiological count data in the applied mass of crop contents are shown in Table 2. The mean number of bacteria in the crop contents were as follows: *E. coli* 4.0 log, coliforms 4.1 log, and total aerobic bacteria 5.7 log CFU/g of crop contents. Prior research has shown that whole crops contained (in log CFUs) 6.5 total aerobes, 5.0 coliforms, 4.4 *E. coli*, and 5.1 *Campylobacter* (Berrang *et al.*, 2000). Crop contents in the current study were *Campylobacter* negative. Applying crop contents in the amounts of about 9, 54, and 231 mg resulted in significant ($P<0.05$) increases in all bacterial population measured, with the biggest increase being noted for total aerobic bacteria. Gizzard contents contained only 4.6 log CFU/g of total aerobic bacteria (data not shown). Total bacterial counts averaged 2.45, 3.38, and 3.67 log CFU for gizzard content mass of 9.3, 61.0, and 120.0 mg, respectively. The lower total aerobic counts and prevalence of specific bacteria found in the gizzard as compared to the crop is probably due to the effect of lower in the gizzard (Duke, 1986).

Bacterial counts from pre-chill broiler carcasses have been reported to average (log CFU per carcass) 6.3, 5.3, 4.8, and 5.2 for total aerobes, coliforms, *E. coli,* and *Campylobacter*, respectively (Cason and Berrang, 2002; Berrang *et al.*, 2004). These counts were adjusted for the number of samples, ml of rinse used, and halved carcasses in order to calculate the increase in whole carcass bacterial counts due to crop contamination. The addition of applied crop mass (Table 1) was multiplied by the average bacterial count. The total added bacterial load from contamination with 231 mg of crop contents would increase whole carcass counts of total aerobes from 6.3 to 6.4 (log CFU). Coliform and *E. coli* numbers would remain at 5.3 and 4.8 log CFU per carcass. Based on these counts and numbers of bacteria found in gizzard, carcass contamination with visible ingesta does not appear to significantly increase bacterial load. These results agree with Bilgili *et al.* (2002), who reported no correlation between carcasses contaminated with visible ingesta and microbial contamination.

Conclusions: A hyperspectral imaging system was used to detect pre-chilled broiler carcasses contaminated with known mass of gizzard and crop contents. The imaging system identified 100% of the 10, 50, and 100 mg gizzard and crop content contaminants applied on the carcasses. Detection of pixels known to be contaminated with gizzard and crop content averaged 53 and 72%, respectively. Improved ground truth pixel accuracy reported in the present study was due in part to a combination of hardware and software changes that have improved the overall performance of contaminant detection. Applying crop contents at increase known amounts resulted in increases of all bacterial population measured. However, bacterial numbers on whole carcasses due to crop contamination were not different. Therefore, the presence of visible ingesta on pre-chill carcasses does not influence microbial counts.

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