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Detection of Septicemia in Chicken Livers by Spectroscopy[1]

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ABSTRACT To establish a procedure for differentiating normal chickens from chickens with septicemia/toxemia (septox) by machine inspection under the Hazard Analysis and Critical Control Point-Based Inspection Models Project, spectral measurements of 300 chicken livers, of which half were normal and half were condemned due to septox conditions, were collected and analyzed. Neural network classification of the spectral data after principal component analysis (PCA) indicated that normal and septox livers were correctly differentiated by spectroscopy at a rate of 96%. Analysis of the data established 100% correlation between the spectroscopic identification and the subset of samples, both normal and septox, that were histopathologically diagnosed. In an attempt to establish the microbiological etiology of the diseased livers, isolates from 30 livers indicated that the poultry carcasses were contaminated mostly with coliforms present in the environment, hindering the isolation of pathogenic microorganisms. Therefore, to establish the cause of diseased livers, a strictly aseptic environment and procedure for sample collection is required.

(*Key words*: poultry inspection, septicemia/toxemia, near-infrared spectroscopy, principal component analysis (PCA), neural network)

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INTRODUCTION

Poultry inspection in the United States began as early as 1926, arising from the need to protect public health from unwholesome meat and poultry. The Poultry Products Inspection Act became law in 1957, requiring inspection of individual birds before and after slaughter and during processing. The Wholesome Poultry Products Act of 1968 established federal-state poultry inspection programs requiring each chicken carcass to be visually inspected at the slaughter plant by federal inspectors. Today, the responsibility of poultry inspection lies with the Food Safety and Inspection Service (FSIS) of the USDA to ensure that the nation's commercial supply of meat, poultry, and egg products is safe, wholesome, and correctly labeled and packaged (FSIS, 1984).

Over the years, the USDA/FSIS has modified many old rules and regulations for meat inspection while also establishing new rules. Until 1996, the basis of meat and poultry inspection was antemortem examination of animals followed by organoleptic examination of carcasses. In addition, representative samples of fresh raw meat and poultry were analyzed for microbial contamination and agricultural chemical residues. To reduce the occurrence of pathogens in meat and poultry products and thereby reduce the incidence of illnesses associated with them, USDA/FSIS introduced new regulations in 1996 that required the development and implementation of Hazard Analysis and Critical Control Point (HACCP) programs, a systematic, science-based process control system for food safety under FSIS guidance.

Under the HACCP program, the inspection roles and responsibilities of FSIS shifted from detecting facility and production problems to validating and verifying the production of safe poultry products by the processing plants, and the poultry processors assumed the responsibility of ensuring product wholesomeness (FSIS, 1996). A new project called HACCP-Based Inspection Models Project (HIMP) was begun in a few plants. HIMP requires zero tolerance for the two categories related to infectious condition and fecal contamination (food safety categories FS-1 and FS-2), in addition to compliance with standards for other consumer protection categories related to dressing defects and other hazards. Under these models, the heavy inspection workload is shifted to the producers. However,

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[1]Mention of company or trade names is for descriptive purposes only and does not imply endorsement or approval by the USDA to the exclusion of other products that may be suitable.

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Abbreviation Key: AD = azide dextrose; FSIS = Food Safety and Inspection Service; HACCP = Hazard Analysis and Critical Control Point; HIMP = HACCP-based Inspection Models Project; LT = Lowry/tryptone; McC = McConkey; PCA = principal component analysis; septox = septicemia/toxemia; TS = trypticase soy; TT = tetrathionate; Vis/NIR = visible/near-infrared.

FSIS retains the authority to take any steps necessary to ensure that the zero-tolerance standards for FS-1 and FS-2 are met (FSIS, 2000).

In general, modern poultry production methods are scientific and uniformly implemented. Product variation is minimal, and defective product can be removed easily. However, several factors can affect the health and condition of each lot of birds. Examples include production season, farm location, and variations in feed nutrition and other husbandry practices among growers. The six major defect categories for which chicken carcasses are removed from the processing line are septicemia/toxemia (septox), cadaver (usually a result of improper slaughter), bruise, tumor, air sacculitis, and ascites. Septox carcasses are often dark red to bluish in color, dehydrated, stunted, or edematous (USDA, 1999).

Large poultry plants slaughter between 200,000 and 400,000 birds per plant per day, and some plants have increased line speeds from 70 to 91 birds per minute. Inspectors working under these conditions tend to develop repetitive motion injuries and attention and fatigue problems. Machine inspection systems that can accurately detect unwholesome carcasses on-line in processing plants would improve the speed, accuracy, and overall effectiveness of the inspection process and could minimize human error and variability while reducing operational costs.

In order to assist the poultry industry with machines that can aid in inspection, several spectroscopic techniques and machine vision systems have been developed at the Instrumentation and Sensing Laboratory. The developments include visible and near-infrared (Vis/NIR) spectroscopy techniques to classify wholesome, septicemia, and cadaver carcasses (Chen and Massie, 1993); Vis/NIR spectroscopy for on-line separation of wholesome and unwholesome carcasses (Chen et. al., 1998); Vis/NIR two-dimensional correlation for the spectral features of chicken breast meat under various conditions for the detection of unwholesome poultry carcasses (Liu and Chen, 2000, 2001a,b; Liu et al., 2000); and image processing for detection of lesions commonly found in viscera (Chao et al., 1999) and pathological changes in poultry hearts due to disease (Chao et al., 2000).

This research is an attempt to use Vis/NIR spectroscopy to analyze and record physical and chemical changes in the livers of septox poultry that are to be rejected under the FS-1 criteria. Based on the spectral data of the liver, the research would establish pattern recognition for both the normal and the septox livers that could be used for machine inspection of poultry. Thereby, identification of the septox birds could be achieved by machine inspection of the liver alone.

MATERIALS AND METHODS

Collection of Livers from Normal Chickens

The birds used in this study originated from different farms and were 6-to-8-wk-old fryers weighing between 1.8 and 2.2 kg. Postmortem examination of carcasses and viscera was performed by the plant veterinarian at the usual inspection station (before the chiller) along the processing line. The general condition, color, and texture of the carcass and gross appearance of the liver, gall bladder, spleen, heart, and lungs were noted. The organs (liver, spleen, heart, and lungs) from each bird were collected in a sterile plastic bag and stored on crushed ice in a plastic cooler. The samples were brought to the laboratory and analyzed within 4 h. The number of samples collected on each date is presented in Table 1.

Collection of Livers from Chicken Condemned Due to Septox and Other Causes

Under the direction of the plant veterinarian, birds unfit for human consumption due to septox or any other causes are usually immediately condemned or hung separately for further examination. For our study, only birds presenting signs of septox were selected. The size, color, muscle and skin conditions of the carcasses, and the gross pathological changes primarily in the liver and also in the other organs were noted. The organs (liver, spleen, heart, and lungs) from each bird were collected in a separate sterile bag and stored on crushed ice in a plastic cooler. The nonliver organs of each chicken were included to aid in the diagnosis of its condition. The samples were brought to the laboratory and analyzed within 4 h. The number of samples collected on each date is also presented in Table 1.

Sample Analysis

Spectral data from the livers of the normal and septox birds were analyzed as described below. However, to establish correlation between lesion due to pathogen involvement and pathological changes in the liver, microbiological and histopathological studies of some of the samples were performed. The number of samples analyzed by each method is presented in Table 2.

Gross Pathology. The general condition, color, and texture of the carcass and gross appearance of the liver, gall bladder, spleen, heart, and lungs were noted for normal and septox carcasses. The normal carcasses from all batches had a uniform yellowish tinge, whereas the livers showed slight variations in size and color. The size, color, muscle, and skin condition of the septox carcasses and the gross pathological changes primarily in the liver (Gross, 1978) and other organs were noted. The pathological changes included inflammation, hemorrhage, enlargement, atrophy, fatty degeneration, extreme variations in color due to toxic condition, formation of membrane on liver surface, and gelatinous deposit on liver.

Microbiology. To establish a microbiological association between the gross pathology and the spectra, 10 normal livers and 20 septox livers were analyzed according to USDA procedures (Rose, 1998; Sharar and Rose, 1998). After the liver surface was sterilized with

TABLE 1. Number of samples collected at various times

Trial	Month	Nominal bird weight (kg)	Number of livers collected	
			Normal	Septicemia
1	August	1.8	5	13
2	September	1.8	6	14
3	September	1.8	4	15
4	October	1.8	3	10
5	October	1.8	7	10
6	December	2.3	30	0
7	December	2.3	30	0
8	December	2.3	16	17
9	December	1.8	6	20
10	January	1.8	14	12
11	February	2.3	15	10
12	March	2.3	14	29
		Average weight = 2.1 kg	150 total	150 total

iodine, a piece (4 to 5 mm) of the liver tissue from inside the organ was transferred to 9 mL of trypticase soy (TS) broth and incubated for 18 h at 37 C. After incubation, a loopful from the broth was plated on to a McConkey (McC) agar plate. Then, 1 mL of the TS broth was also transferred to each of the following, in triplicate: 9 mL of tetrathionate (TT) broth, 9 mL azide dextrose (AD) broth, and 9 mL of Lowry/tryptone (LT) broth in triplicate. The McC plates and LT, TT, and AD tubes were incubated 18 h at 37 C. After incubation, 1 mL was transferred from LT broth to 9 mL of *E. coli* broth with 4-Methylumbellifryl B-D-glucuronic acid (EC-MUG), and incubated at 37 C for 18 h. A loopful from each TT broth tube was plated on to xylose lysine turgitol-4 (XLT-4) agar, and after incubation at 37 C for 18 h, plates were examined for typical *Salmonella* colonies.

Spectral Measurement. A circular section of liver was cut from one lobe using a circular core cutter (4-cm diameter). The liver section was placed inside the sample cup with the top surface of the liver against the glass window. Any excess liver sample tissue (i.e., for a very thick sample) exceeding the sample cup volume was cut away so that the bottom surface of the sample was flush with the rim of the sample cup. A round glass cover was then placed over the liver sample to hold it in the sample cup. The sample cup was placed in the NIRSystems 6500 spectrometer[3] and the sample spectrum was measured. Each spectrum was an average of 32 scans at 2-nm intervals between 400 to 2,500 nm. In the spectrometer, source illumination is directed at the sample through the glass window, and fractions of the light will be absorbed or reflected by the sample material. A detector measures the amount of reflected light at each 2-nm interval, and the absorbance spectrum is recorded as $\log_{10}(1/R)$ where R is the ratio of the reflected light to the incident light from the source upon the sample.

Spectral characteristics in the visible spectrum, between 400 and 700 nm, give information about the pigment characteristics of myoglobin and other tissue components. The near-infrared region, between 700 and 2,500 nm, shows spectral characteristics arising from specific chemical bonds and their vibrations in the molecules of the tissue. For example, in Figure 2, absorbances at 1,440 and 1,940 nm are overtones of the fundamental water OH stretching vibration at longer wavelengths (Murray and Williams, 1987).

Spectral Processing and Analysis. A model for classifying normal and septox livers has been developed. It involves pretreating the spectra with an offset and a second difference algorithm to remove baseline effects. Then the pretreated data set is subjected to principal components analysis (PCA), where each spectral vector in the wavelength space is transformed into a lower dimensional factor space. The factors (also called eigenvectors or loadings) are mutually orthogonal (uncorrelated). The first factor, a vector of spectral reflectance, is chosen to account for the largest possible variance of reflectance in the class. Each successive factor is then chosen to account for the largest possible amount of the remaining variance. The projection values of each spectral vector onto the factors are called scores. Instead of many reflectances in wavelength space, each spectrum can be adequately represented by a few scores in the factor space. Thus, the dimension of the spectra is effectively reduced (Galactic Industries Corporation, 1999; Pimentel, 1979).

The scores from the PCA analysis are then used to train a neural network, which does the classification. Various parameters involved in the pretreatment, PCA, and neural networks were tested and are described in detail by Hsieh et al. (2002).

Each model was trained on 200 samples (100 normal, 100 septicemic) randomly selected from the 300 measured with the spectrophotometer and then tested on the remaining 100. The liver classification results for the best model are reported here. Specific parameters follow. For offset correction, a different constant was subtracted from each spectrum so that each resulting offset spectrum had the same minimum value of zero. For second difference pretreatment, gaps of 2, 8, 15, 31, and 75 were tested, and gap = 31 was optimal. For PCA, the number of scores equal to 60 was found optimal after testing 5, 15, 30, 60,

[3]FOSS NIRSystems, Silver Spring, MD.

TABLE 2. Number of liver samples used for each analysis or measurement

Sample type	Gross pathology	Spectroscopy	Histopathology	Microbiology
Normal	150	150	28	16
Abnormal	150	150	72	33

and 90. The neural network used was a feed-forward back-propagation network with 60 input nodes (receiving the 60 scores), one hidden layer with 30 nodes, and two output nodes. The target values of the output nodes were (1,0) for normal and (0,1) for septicemic livers. The underlying equations and parameter definitions are given by Hsieh et al. (2002).

Histopathology. To confirm an association between the changes in the liver spectra with the pathological changes in the liver, one lobe of each liver (a total of 99 = 28 normal + 71 septox) from each of four lots collected on four different days was transferred into a jar with formaldehyde. The jars were sent to FSIS Eastern laboratory in Athens, Georgia, for histopathological study, to detect pathological changes in each of the liver samples.

RESULTS

Gross Pathology

Normal livers varied in size from 7.6 cm × 5.1 cm to 8.9 cm × 6.5 cm. They had smooth surfaces and firm texture, but the colors varied among lots from light to deep brown to chocolate to magenta.

The size variation in septox livers was greater than that of normal livers, ranging from 3.8 cm × 3.8 cm to 10.2 cm × 12.7 cm. Some septox livers presented only one notable lesion or change, but several livers had multiple changes. The apparent syndromes noted in the total of 150 septox livers were as follows: 81 enlarged, 50 pliable or fragile, 37 congested, 30 evidence of hemorrhage, 17 covered with

TABLE 3. Reactions of organisms isolated from abnormal livers on various media previously cultured in trypticase soy broth

Sample number[1]	McC[2] +	McC −	LT	EC	EC-MUG	AD	BHI + 6.5% NaCl	BHI at 45 C	mEnt	
N118	+	−	+	+	+	+	+	+	−	
N119		+	−	+	+	+	+	−	−	−
N120	+	−	+	+	+	+	+	+	+	
N121	+	+	+	+	+	+	+	+	+	
N122	−	−	−	−	−	+	−	+	−	
N123	+	+	+	+	−	+	−	+	+	
N124	+	+	+	+	−	+	+	+	+	
N125	+	+	+	+	−	+	−	−	−	
N126	−	+	+	−	−	+	+	+	+	
N127	+	+	+	+	+	+	−	+	−	
S243	+	−	+	+	−	+	+	+	−	
S244	+	−	+	+	+	+	+	+	−	
S245	+	−	+	+	+	+	+	+	+	
S246	+	+	+	+	+	+	+	+	+	
S247	+	+	−	+	+	+	+	+	−	
S248	+	−	+	+	−	+	+	+	+	
S249	−	+	+	+	−	+	+	+	+	
S250	+	−	+	+	+	+	+	+	−	
S251	+	−	+	+	+	+	−	−	−	
S252	+	−	+	+	+	+	−	−	−	
S253	+	−	+	−	+	+	−	−	−	
S254	+	+	+	+	+	+	+	+	+	
S255	+	−	+	+	+	+	+	+	+	
S256	+	+	+	−	+	+	+	+	+	
S257	+	−	+	−	+	+	−	+	−	
S258	+	−	+	+	+	+	−	+	−	
S259	+	−	+	+	+	+	+	−	−	
S260	+	+	+	+	+	+	+	+	+	
S261	+	−	+	−	−	+	−	−	−	
N262	+	−	+	−	+	+	−	+	−	

[1]N = normal liver; S = septicemia/toxemia liver.

[2]McC+ = McConkey agar with lactose; McC– = McConkey agar without lactose; LT = laurel tryptose broth; EC = *Escherichia coli* broth; EC-MUG = *E. coli* broth with MUG (4-Methylumbellifryl B-D-glucuronic acid; AD = azide dextrose broth; BHI+6.5% NaCl = brain heart infusion broth with 6.5% sodium chloride; BHI = brain heart infusion broth incubated at 45 C; mEnt = modified enterococcus agar. All samples analyzed for *Salmonella* and *E. coli* were negative. All (colony-forming units on xylose lysine turgitol-4 (XLT) agar were yellow.

TABLE 4. Confusion matrix for classification of 100 livers [50 normal, 50 septicemia/toxemia (septox)] using second difference method with gap = 31 and 60 principal component analysis scores

	Predicted normal	Predicted septox	Type I error (%)
Actual normal	49	1	2
Actual septox	3	47	6
Type II error (%)	5.8	2.1	

gelatinous material or a membrane, 12 necrotic, 2 mottled, 1 pulpy or gritty texture, and 1 cyanotic. The colors displayed by these livers varied from oxblood red to blue to black.

Microbiology

The typical colonies from TS and LT broths isolated on McC plates from normal or septox liver samples cultured in enrichment media or selective media were almost pure cultures of gram-negative bacillus, probably a coliform (Table 3). None of the organisms isolated were confirmed as salmonella or streptococcus. Only one sample from TS broth when plated on McC agar produced four to five tiny, gray-white hemolytic colonies. The isolate could not be correlated with the gross pathology of the septox liver. Presumably, the isolated organisms were not septox-related contaminants in the sample.

Spectroscopy

The spectra of eight normal and 10 septox chicken livers are shown in Figures 1 and 2, respectively. (The discontinuity at 1,100 nm arises from the switch between two different detectors used to detect the wavelength ranges below and above that point.) In order to see the differences more clearly, averages of normal and septox spectra are shown in Figure 3. Also shown are averages of the offset spectra; each spectrum was offset by setting $\log_{10}(1/R)$ at the spectral minimum (1,100 nm) to zero and adjusting the entire spectrum accordingly with that same shift. The pattern of the offset averages of the normal and septicemia liver spectra appear to be different. Notable spectral differences between normal and septox livers were found at 750 and 2,300 nm.

By using the best model described above, a training set of 200 samples, and a testing set of 100 samples, the testing results in Table 4 were obtained. A correct classification rate of 96% breaks with 98% accuracy for normal samples and 94% for septicemic. The corresponding type I errors were 2 and 6%. The first results in economic loss, as the normal samples would be condemned. The second results in health risk, as the septicemic samples would be passed as normal. In practice, a suitable adjustment of thresholds would reduce the incidence of passed septicemic carcasses to zero, as required by FS-1. The passed, normal birds would be diverted to a line not receiving bird-by-bird inspection. The resulting increase in the number of falsely condemned birds would be counteracted by bird-by-bird inspection of the carcasses remaining on the original line. Thus, the job of organoleptic bird-by-bird inspection would change in character from finding a few septicemic birds on a predominantly normal line to finding a few normal birds in a predominantly septicemic line.

Histopathology

Histopathological tests were conducted for 99 liver samples (28 normal, 71 septox). All 28 normal livers were identified to be without pathological syndrome. All 71 liver samples from birds rejected due to septox were identified by microscopic examination as having pathological changes in the liver tissue. The changes in the liver tissue were diagnosed as chronic pericholangitis, cholangiohepatits, edema, hepatic fibrosis, necrosis, necrotizing hepatitis, toxic hepatitis, congestion, lipidosis, and kuffer cell hyperplasia. More than one-half of the livers presented more than one change in the tissue.

Of the 71 septox samples, 48 were included in the training set and 23 in the testing set. Of the 28 normal samples, 19 were included in training and 9 in testing. The neural network classification model based on the liver spectral data correctly identified the 23 septox and 9 normal samples included in the testing set in Table 5.

DISCUSSION

This study indicates that determining the etiology of a septox condition is a complicated task. Contamination of liver due to the environmental and normal processing conditions makes pinpointing the etiology of a disease condition difficult. In case it becomes necessary to isolate pathogens causing pathological changes in the liver with the potential to cause human health risk, aseptic sampling procedures will be needed before the birds enter the routine processing line.

TABLE 5. Prediction of histopathologically analyzed samples by neural network model using second difference method with gap = 31 and 60 principal component analysis scores

		Neural network model training and prediction			
	Total samples	Training samples	Testing samples	Correctly predicted testing samples	Error (%)
Normal	28	19	9	9	0
Septox[1]	71	48	23	23	0

[1]Septicemia/toxemia.

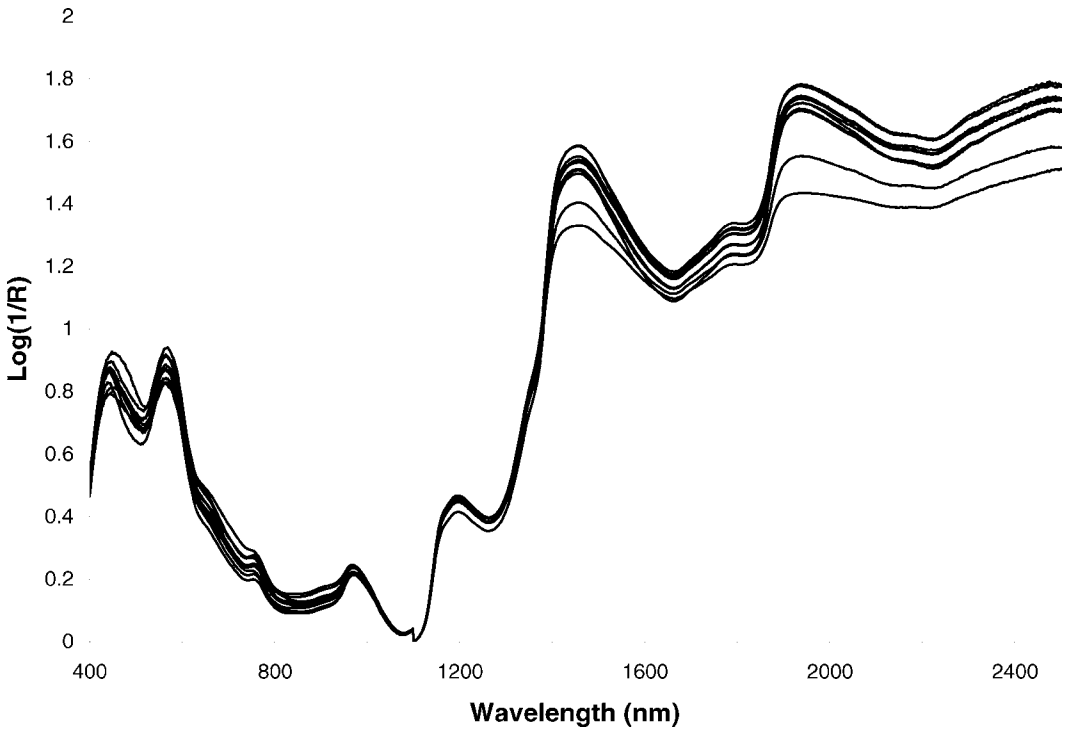

FIGURE 1. Offset-corrected spectra of eight livers from eight normal chickens.

Variability in the weight of chicken carcasses supplied by different farms at different times of the year was evident. The sizes and colors of the corresponding livers reflected this variation. These variations might have resulted from seasonal changes, minor variability in the husbandry practices, and variations in the feed. This study has shown that spectroscopic examination of livers can be made with 95% accuracy compared to organoleptic

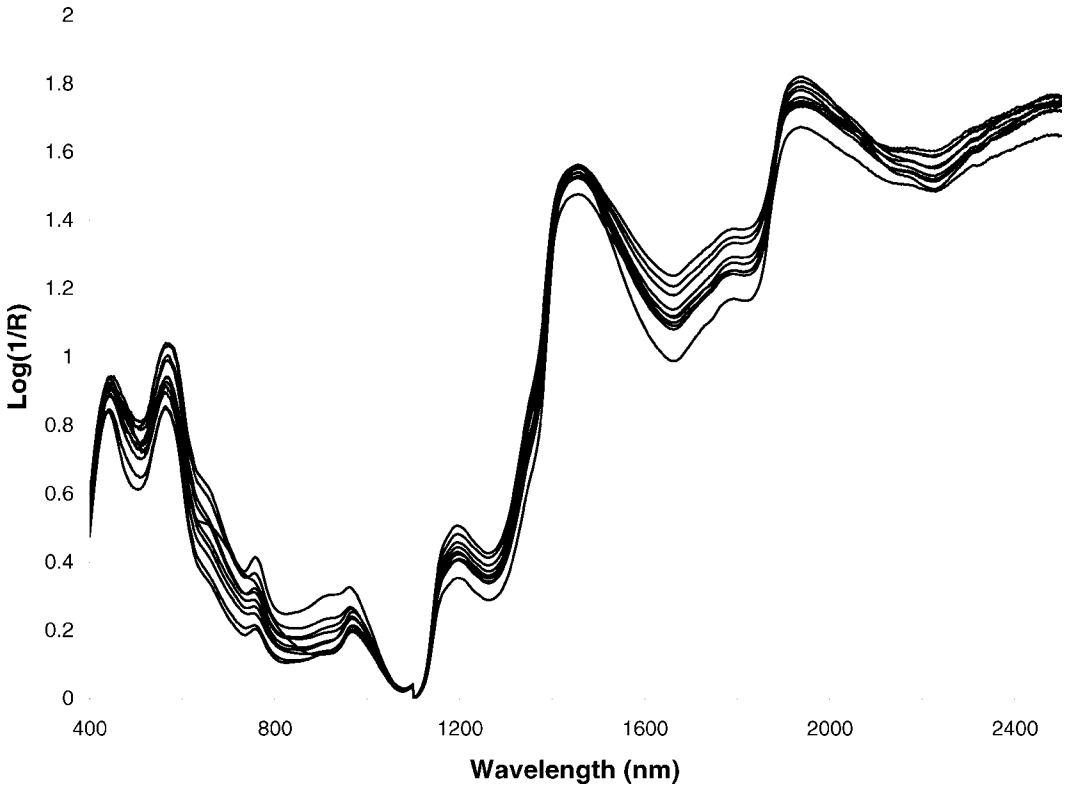

FIGURE 2. Offset-corrected spectra of ten livers from ten septox chickens.

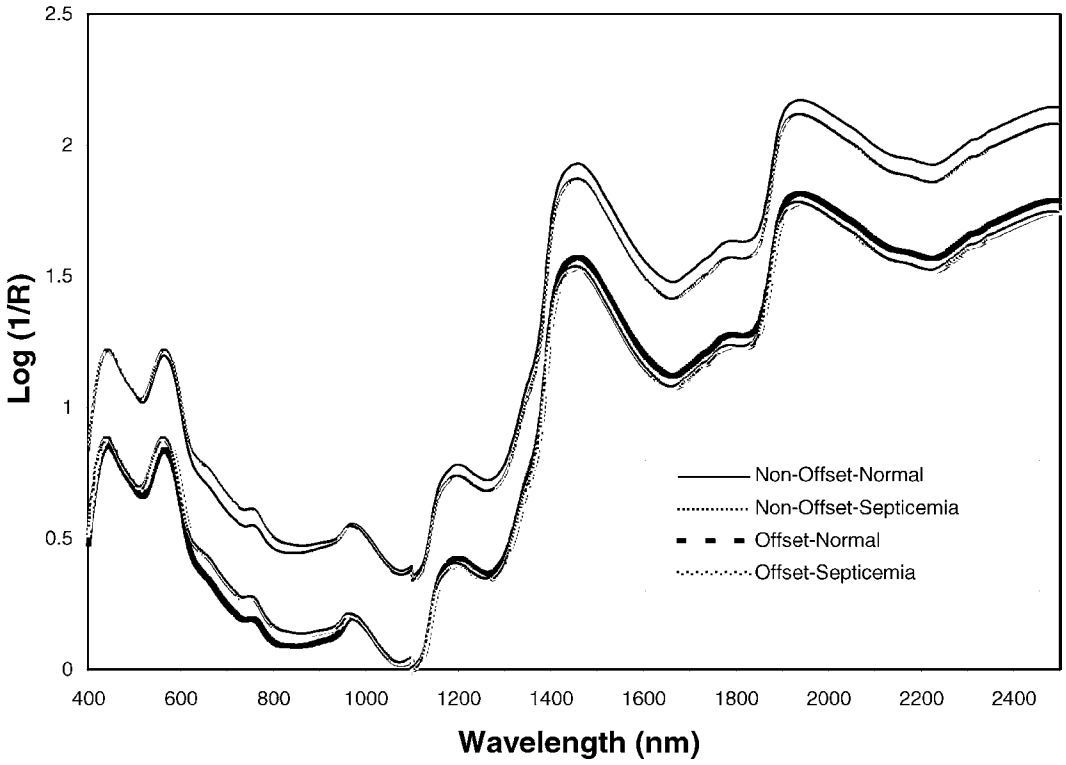

FIGURE 3. Average offset-corrected spectra and average uncorrected spectra of normal and septicemia/toxemia (septox) chicken livers.

examination of livers. With proper implementation for real-time acquisition of liver spectra, this method could have great potential for automating the screening of carcasses for the FS-1 condition of septox.

It seems that the septox spectroscopic method here is limited to the gross determination of the septox condition from the liver spectra. Considering the successful identification of 98% of the normal livers by spectroscopy, the other 2% of normal livers might have had very early stages of tissue change that were invisible to the human eye and also beyond the capacity of spectroscopic identification as well. Further research will try to correlate spectroscopic information to microbiological determinations rather than solely organoleptic determinations. Even with the size and color variations, the 94% detection rate of septox livers is encouraging.

A few chicken processing plants are testing the viscera pack system whereby the viscera (including the liver) are separated from each bird, but are kept on a tray that moves along the line with the corresponding carcass. As this practice becomes more commonly adopted, an automated liver inspection system will become more easily implemented and beneficial to the industry. Implementing an automated liver inspection system with the newly developed automated poultry carcass inspection system at Instrumentation and Sensing Laboratory will greatly improve the FSIS Poultry Inspection Program.

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