Chapter 18
Analysis of Decomposition Fluid Collected from Carcasses Decomposing in the Presence and Absence of Insects

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Abstract Most decomposition studies investigate soft tissue degradation in the presence of insects, however several studies have shown that when insect activity is excluded from carcasses, the rate of decomposition slows down. The goal of this study was to explore the effect of insect activity on the chemical properties of decomposition fluid. Fluid was collected from pig (*Sus scrofa)* carcasses over the course of two summer trials (2011 and 2012) conducted in southern Ontario, Canada. The pH and conductivity were measured and fatty acids were analysed using Attenuated Total Reflectance- Infrared (ATR-IR) spectroscopy. Results were compared between insect inclusion, partial exclusion, and complete exclusion carcass groups. The results indicate that the presence of insects increases the pH and decreases the conductivity of decomposition fluid. Spectral fatty acid results did not appear to vary greatly between experimental groups. The overall levels were not sufficiently different between carcass groups to conclude that the presence of insects played an important role in the fatty acid degradation process.

18.1 Introduction

Decomposition, when allowed to progress to completion, will result in the complete disintegration of soft tissue, leading to partial or complete skeletonisation (Evans 1963; Dent et al. 2004; Goff 2009). Soft tissue can be biochemically degraded

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through autolysis and putrefaction (Gill-King 1997; Dent et al. 2004; Janaway et al. 2009), or physically removed by the feeding of vertebrate and invertebrate scavengers (Amendt et al. 2007; O'Brien et al. 2007; Goff 2009). Decomposition can be characterized using different stages, but the process is typically categorized using the stages first established by Payne (1965): fresh, bloat, active decay, advanced decay, dry, and remains.

There are many factors that influence the rate of decomposition, however studies have found that when temperature is accounted for, insects play the most significant role (Simmons et al. 2010a). In addition, it has been observed that carcasses decompose at a significantly accelerated rate in the presence of insects when compared to those which are excluded from insect activity (Simmons et al. 2010a). Few studies have explicitly investigated the decomposition process in the absence of insects. The first study to do so was conducted by Payne (1965), where foetal pig carcasses were used to monitor the progression of decomposition. Payne (1965) observed that these carcasses exhibited different decomposition patterns from those colonized by insects, and established an alternative set of decomposition stages to characterize the process: fresh, bloating and decomposition, flaccidity and dehydration, mummy stage, and desiccation and disintegration. It was also observed that the carcasses decomposed more slowly than their insect-colonized counterparts (Payne 1965). It was hypothesized that insects accelerate the rate of decomposition by distributing bacteria throughout the carcasses in the fluids they secrete, and through mechanical burrowing as they feed (Payne 1965).

In a more recent study, the effect of insect exclusion on the decomposition process was investigated using rabbit carcasses that were either buried or deposited on the ground surface and protected by screens (Simmons et al. 2010b). The results showed that the most important influence on the rate of decomposition, when time and temperature were accounted for using accumulated degree days (ADD), was the presence of insects (Simmons et al. 2010b). The exclusion carcasses were observed to decompose slower than those accessed by insects, regardless of the method of exclusion (Simmons et al. 2010b).

The study of decomposition-related products, such as soil, mammalian soft tissue, and fluid, is a prominent focus of researchers who study cadaver decomposition. One of the main goals in studying these decomposition products is to develop methods that identify chemical biomarkers that can be used to estimate post-mortem interval (PMI) or correlate the findings with specific decomposition stages (Swann et al. 2010c).

Many studies have concentrated on identifying potential chemical biomarkers in soil and tissue, however few studies have focused on their detection in decomposition fluid. Decomposition fluid is a challenging matrix to study since it is often a complex chemical mixture that contains insects, microorganisms, and other debris from the surrounding environment (Swann et al. 2010c). Previous research using decomposition fluids has focused on examining short chain volatile fatty acids and long chain fatty acids from pork rashers, stillborn piglets, and adult pig carcasses (*Sus scrofa*) in an attempt to determine their utility in estimating PMI (Swann et al. 2010a, b). The same authors also developed a method using capillary zone electrophoresis to detect selected biogenic amines and amino acids in decomposition fluid (Swann et al. 2010d).

Adipose tissue is composed of 60–85 % lipids, 90–99 % of which are triglycerides (Reynold and Cahill 1965). During decomposition, the degradation of lipids results in the hydrolysis of the triglycerides and other neutral lipids (Fiedler and Graw 2003; Dent et al. 2004; Forbes et al. 2005b), including diglycerides and phospholipids (Kramer and Hulan 1978). Intrinsic lipases work to free the fatty acids from the glycerol backbone, leading to a mixture of free saturated and unsaturated fatty acids (Dent et al. 2004; Forbes et al. 2004; Janaway et al. 2009; Notter et al. 2009). Following hydrolysis, anaerobic bacteria present within the body will promote hydrogenation of unsaturated fatty acids, thus converting them into their saturated counterparts (Evans 1963; Notter et al. 2009). It is therefore expected that, during decomposition, the levels of free fatty acids in the body will initially increase with the hydrolysis of neutral lipids, shown by an increase in saturated fatty acids and a concomitant decrease in unsaturated fatty acids. The examination of fatty acid degradation products in decomposition fluid therefore has the potential to demonstrate a correlation with the post-mortem period which may be valuable in estimating post-mortem interval.

Insects, like other living organisms, contain fatty acids to serve various biological purposes. Most of their fatty acids, as in mammals, are bundled in triglycerides for metabolic energy (Stanley-Samuelson et al. 1988). They contain many of the major and most commonly reported fatty acids: palmitic, palmitoleic, stearic, oleic, linoleic, and linolenic, which account for approximately 98 % of the fatty acids in whole lipid extracts (Stanley-Samuelson and Dadd 1983). More specifically, the dominant fatty acid detected in triglyceride fractions of Diptera is palmitoleic acid (Fast 1966; Stanley-Samuelson et al. 1988) and more than 50 % of the fatty acids in neutral lipids are less than 18 carbons long (Fast 1966). Long chain polyunsaturated fatty acids are also a regular component of insect tissues, but they are mainly found in phospholipid fractions (Fast 1966; Stanley-Samuelson and Dadd 1983; Stanley-Samuelson et al. 1988). Very little is known about whether insect fatty acids are transferred to remains as they feed, and how insects affect the fatty acid degradation profile of soft tissue, if at all.

The objective of this study was to identify trends in the chemical properties of decomposition fluid, including pH, conductivity, and lipid degradation products, and compare these between insect- included and excluded carcasses to determine their effect on the chemical properties of decomposition by-products.

18.2 Materials & Methods

18.2.1 Experimental Site

The current research was conducted in Oshawa, Ontario, Canada, 68 km northeast of Toronto (43°94 N, 78°90 W). The fenced field site was located in a temperate open grassland. A forest was located outside of the facility on the north side, but did

not provide cover or shade for any of the experimental subjects. This southern Ontario location experiences warm summers (17 °C) and cool winters (−5 °C) (Bernhardt n.d.). The experiments were conducted during the summer months, from June-September 2011 (Trial 1) and 2012 (Trial 2).

18.2.2 Weather Data

A HOBO® Micro Station Data Logger was placed within the confines of the experimental site and recorded hourly temperatures, rainfall, and relative humidity for the duration of the studies. Data was subsequently retrieved from the data logger using HOBOware® Pro Graphing and Analysis Software, Version 3. Temperature data was used to calculate accumulated degree days (ADD), which is calculated by averaging daily temperatures (above 0 °C) and adding it to the summed average of the previous day (Megyesi et al. 2005). The use of ADD as a time scale allows studies conducted in different environments and at different times to be more accurately related to one another by accounting for temperature. It therefore accounts for most of the variability observed between carcasses, in terms of the rate of decomposition (Michaud and Moreau 2011). However, one recent study showed that the use of ADD may not accurately predict the rate of decomposition in certain environments (Myburgh et al. 2013) and thus would prevent comparison of studies between different geographical regions. Additional factors may also influence the rate of decomposition, even when temperature is accounted for in a given environment (Myburgh et al. 2013) hence it must be used with caution. Since the current study was replicated in the same environment and during the same season it was determined to be the most applicable method for comparing data across both trials.

18.2.3 Experimental Set-Up

Pig carcasses (*Sus scrofa domesticus)* were used as human analogues due to the ethical restrictions of using human cadavers for decomposition research in Canada. Pig carcasses are appropriate models for human decomposition because they have a similar internal anatomy, fat distribution, and are relatively hairless (Schoenly et al. 2006). They are also omnivorous, leading to the belief that they will share similar gut flora (Anderson and VanLaerhoven 1996).

Six sub-adult pig carcasses, weighing between 50 and 60 lbs, were used in each trial. All subjects were killed on the same day by captive headbolt, following regulated procedures at a local abattoir. All carcasses were transported to the research facility in sealed storage containers, to prevent the entry of insects. Three carcasses were used in each of the control groups (accessible to insects) and experimental groups (excluded from insects). The carcasses that were accessible to insects were used as controls since most decomposition studies are conducted in outdoor environments where insects are available to aid in the decomposition process. Much

knowledge is therefore available in terms of the expected visible changes carcasses will exhibit when decomposing in an outdoor environment. Any visible differences or differences in chemical properties of collected fluid for the exclusion carcasses placed in the same environment could therefore be attributed to the lack of insect activity, as opposed to other environmental factors.

Each carcass was placed in a clear, plastic container, on top of a plastic resin shelf containing holes. The shelf served to raise the carcasses above the base of the containers so that the carcasses were not decomposing in a pool of decomposition fluids. The holes in the shelves allowed for the fluids to drain away from the carcasses. The containers were also placed on a slight angle, to allow the fluids to drain to one side for collection. The containers in which the control carcasses were placed were shallow ($34\frac{5}{8}'' \times 18\frac{3}{4}'' \times 7''$), to allow the full exposure of the carcasses to available insects. The experimental carcasses were placed into deeper containers ($34\frac{5}{8}'' \times 18\frac{3}{4}'' \times 12\frac{1}{2}''$), to prohibit accessibility of insects. A double layer of mosquito netting was placed over the containers and sealed with Velcro along the perimeter of the opening to prevent the entry of insects for the experimental carcasses. All carcasses were protected from vertebrate scavengers by placing 3 ft × 2 ft × 1 ft cages with 1 cm mesh over the containers. The cages used to protect the experimental carcasses were also covered with another layer of mosquito netting to further inhibit the entry of insects. Petroleum jelly was used along the bottom perimeter of the containers to prevent the entry of crawling insects.

18.2.4 Sampling Regime

Photographs and observations were collected once a day each sampling day, beginning the end of May (2012, Trial 2) or beginning of June (2011, Trial 1). Fluid was also collected once daily on each sampling day. Serological pipettes attached to a vacuum bulb were used to collect available fluid. A new pipette was used for the collection of fluid from each carcass. If fluids were too viscous to collect with pipettes, scoopulas were used to collect the fluid. The scoopulas were rinsed with denatured alcohol between samples. Collected fluid was placed in glass jars and stored at −4 °C until analysis. Any remaining fluid in the containers was removed using siphons. This was to prevent the mixing of fluids released at different time periods and to ensure an accurate chemical degradation profile was determined for each day of decomposition. The frequency of fluid collection depended on the rate of decomposition and the presence of fluid in the containers.

The exclusion of insects was further necessary during visual observations and sample collection of the experimental subjects. A mosquito netting-lined tent was placed over the researcher and the carcass following removal of the anti-scavenging cage. The double layer of mosquito netting that covered the containers was only removed on one corner of the container to allow for the collection of fluid. Any Diptera that entered the tent were subsequently removed. Once fluid collection was complete, the netting and cage were promptly replaced over the container.

Table 18.1 Description of decomposition stages used to characterize the inclusion carcasses

Decomposition stage	Characteristics
Fresh	No discolouration, minimal change
Bloat	Inflation of the abdomen and raised limbs
	Marbling/skin discolouration
Active decay	Extensive maggot feeding, formation of masses
	Strong ammonia odour
	Exposed skin appears leathery and may be discoloured (orange-red)
Advanced decay	Little cadaveric tissue remains
	Few insects present on the carcass
	Some exposure of bones
	Gradual loss of moisture from remaining tissue
Dry	Dry skin, cartilage, and bones
	Bones may appear dirty
Remains	Only hair, parts of skin, bones, and teeth remain
	Teeth and bones are bleached

18.2.5 Stages of Decomposition

The six stages of decomposition initially established by Payne (1965) were used to characterize the process among the inclusion (control) carcasses. The characteristics used to identify each stage can be found in Table 18.1. The intent of the experimental group was to completely exclude insects for the entire period of study however insects did eventually gain access to most of the carcasses in the experimental group. Delayed colonization was observed among these carcasses, compared to the controls, and since access was limited, noticeably fewer insects were able to feed on the available soft tissue (i.e. they were unable to completely colonize the carcass and were restricted to feeding where they initially gained access). Succession patterns were not observed because attempts were continually made to prevent the entry of insects, even after they gained access. These carcasses were subsequently called partially-excluded carcasses and five stages were used to characterize the decomposition process (Table 18.2). Only one carcass remained completely excluded from insects for the period of study and four stages were used to describe the decomposition process of this carcass (Table 18.3). Different stages of decomposition were required to categorize each set of carcasses due to the variable processes observed.

18.2.6 pH & Conductivity Analyses

Approximately 3 mL of fluid was transferred to disposable culture tubes for pH and conductivity analysis. The pH values were measured using a digital pH meter (Denver Instrument, Ultra Basic pH Meter, Bohemia, NY). The pH meter was calibrated

Table 18.2 Description of decomposition stages used to characterize the partially excluded carcasses

Decomposition stage	Characteristics
Fresh	No discolouration, minimal change
Bloat	Inflation of the abdomen and raised limbs
	Marbling/skin discolouration
Localized tissue removal	Few, distinct maggot masses (mainly in the head and dorsal regions)
	Exposed skin appears leathery and may be discoloured (orange-red)
	Ammonia odour
Dry decomposition	Large amount of soft tissue remains (body form still relatively intact)
	Skin appears dry and leathery, but underlying tissues retain moisture
	Microbial decomposition still occurring
	Maggots have migrated and there is no further insect activity
	Few bones exposed, if any
Desiccation	Overall dry appearance
	Skin and underlying tissues dry; skin is hard
	Exposed bones may become bleached

Table 18.3 Description of decomposition stages used to characterize the completely excluded carcass

Decomposition stage	Characteristics
Fresh	No discolouration, minimal change
Bloat	Inflation of the abdomen and raised limbs
	Marbling/skin discolouration
Deflation	Deflation of abdomen, limbs fall limp over belly
	Carcass begins to flatten
	Tissues retain moisture
	Odours similar to domestic livestock
Dry decomposition	Substantial amount of soft tissue present (body form still intact)
	Dry skin and underlying tissue
	Microbial decomposition still occurring
	Hair remains on almost entire body
	Some skin discolouration

using buffer solutions with pH values of 4, 7, and 10 (Fisher Scientific, New Jersey, NY) prior to measuring the pH of the samples. The conductivity measurements were carried out using a digital dual pH-conductivity meter (Mettler-Toledo Seven Multi conductivity meter, Switzerland). The conductivity meter was calibrated using a 0.01 M KCl solution (>95 % purity, Fisher Scientific, New Jersey, NY).

18.2.7 Infrared Spectroscopy

One to five mL of fluid was transferred to 20 mL centrifuge tubes, depending on the degree of dilution resulting from precipitation. Lipids were extracted from fluid samples using a modified Folch method (Stuart et al. 2005) for analysis using infrared spectroscopy. Six mL of a chloroform-methanol (2:1 v/v) solution (both HPLC grade, Fisher Scientific, New Jersey, NY) was added to each tube and sealed. Samples were placed in a FS110D Sonicator (Fisher Scientific, Waltham, MA) for three 10 min intervals. One mL of deionized water was added to each of the samples, followed by 5 min of centrifuging at 3000 rpm. The organic layer from each sample was transferred to scintillation vials by pipette and subsequently dried using a Savant SC210A speedvac concentrator, attached to an RVT4104 refrigerated vapour trap (Thermo Electron Corporation, Madison, WI). Samples were either analysed immediately or stored at −4 °C until analysis. The concentrated product was directly applied to the germanium ATR crystal of a Nicolet 4700 Fourier transform infrared spectrometer, equipped with an ATR Smart Performer (ThermoFisher Scientific, Waltham, MA) for analysis. The spectra were scanned over the frequency range of 4000–500 cm^{-1}, with a resolution of 4 cm^{-1}. One twenty eight scans were recorded and collected using Omnic software. Relative band absorptions were calculated using the saturated C-H stretching band (2926–2913 cm^{-1}) as the reference band.

18.3 Results

18.3.1 Weather Data

Average daily temperatures and daily sums of precipitation were calculated for each trial (Fig. 18.1). The mean temperatures experienced during both trials were similar: 20.1 °C in Trial 1 and 19.3 °C in Trial 2. The temperature trends observed were also comparable between the two trials, in that they peaked after approximately 40 days, and then decreased until the end of the study (Fig. 18.1). However, the temperatures were lower for the first week of Trial 2, compared to those of Trial 1 (Fig. 18.1).

Trial 1 experienced a greater amount of precipitation (225.0 mm) than Trial 2 (190.4 mm) overall. However, during the first week of Trial 2 a greater amount of precipitation was recorded (28.4 mm) than in Trial 1 (14.5 mm). For a period of approximately 3 weeks during Trial 1 (from days 28 to 52), no precipitation was recorded (Fig. 18.1), which led to very hot and dry conditions. After this point (mainly between days 53 and 75), the majority of the precipitation for Trial 1 was recorded (Fig. 18.1). In contrast, precipitation regularly occurred throughout Trial 2 (Fig. 18.1).

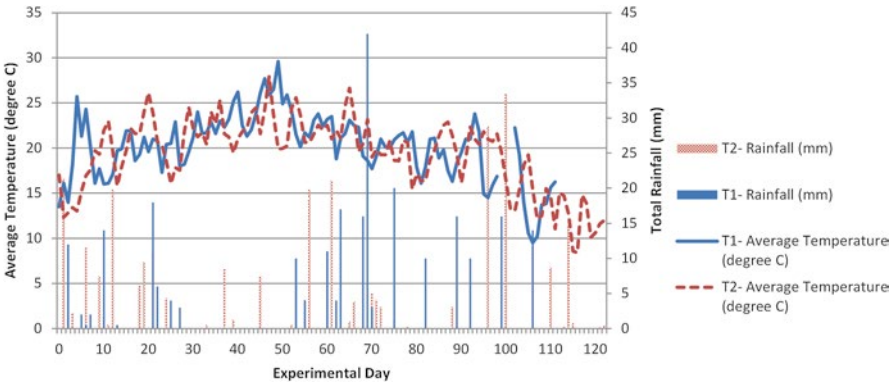

Fig. 18.1 Temperature and rainfall data during Trial 1 (2011) and Trial 2 (2012)

18.3.2 Stages of Decomposition

Although every attempt was made to completely exclude insects during both trials, a limited number of insects did gain access to all experimental carcasses in Trial 1 (on experimental day 7; 153 ADD) and two of the experimental carcasses in Trial 2 (on experimental days 10 and 11; 179 and 202 ADD, respectively) however their presence was localised to specific regions of the carcass (Table 18.2). These carcasses were subsequently referred to as partially excluded. One carcass was completely excluded from insects during Trial 2.

The fresh stage persisted for 2 days (29 ADD) in all carcasses in both trials (Figs. 18.2, 18.3 and 18.4). Following this stage, the patterns of decomposition diverged between carcass groups and the rate of decomposition differed between years (Figs. 18.2, 18.3 and 18.4).

18.3.3 pH Measurements

The pH values in Trial 1 were initially neutral for both the control and partial exclusion groups (Fig. 18.5a). The pH of the partially excluded group gradually increased thereafter, until approximately day 20 (399 ADD; early dry decomposition), after which point the values decreased for the remainder of the study (Fig. 18.5a). The final pH values recorded were approximately 7.5 (Fig. 18.5a). Many of the values for the inclusion group could not be measured due to the inability to collect fluid between experimental days 6–9 (133–178 ADD; active decay) and 13–15 (256–298 ADD; early advanced decay) due to the large numbers of maggots present. It is therefore difficult to determine whether this trend also took place for these samples. Following day 18 (358 ADD), the inclusion carcass values appear lower than those

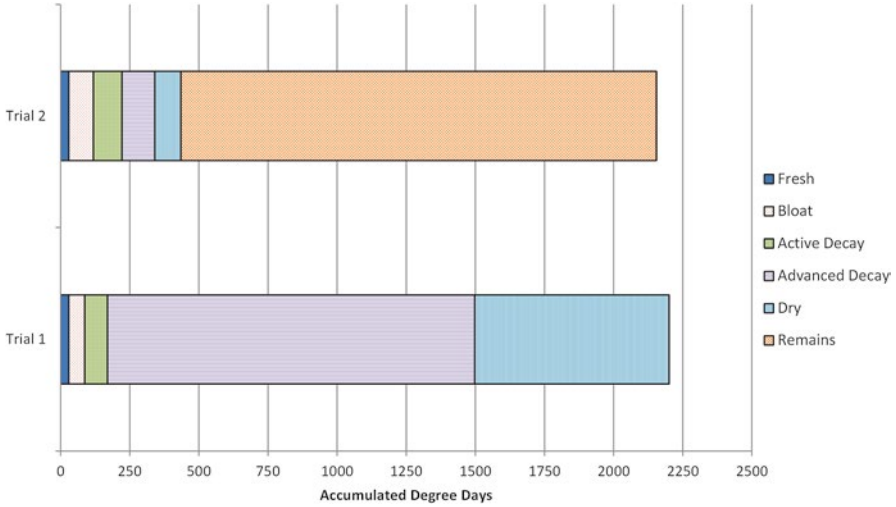

Fig. 18.2 Onset of the decomposition stages for the inclusion carcasses in Trials 1 (2011) and 2 (2012)

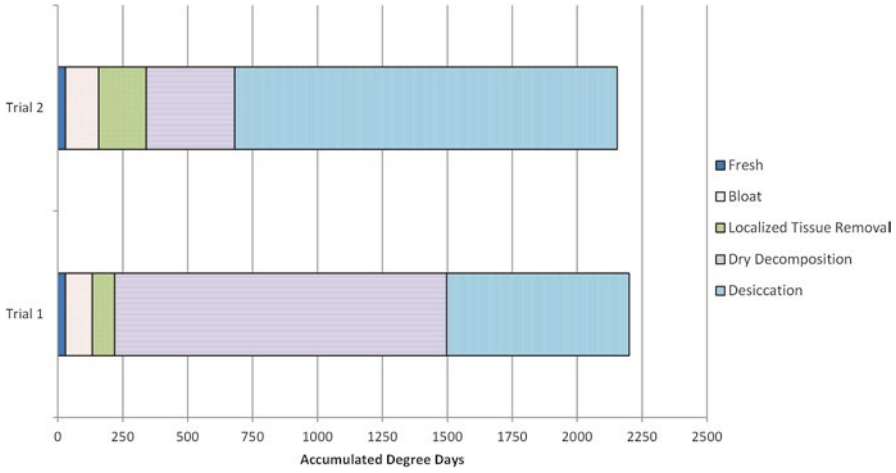

Fig. 18.3 Onset of the decomposition stages for the partially excluded carcasses in Trials 1 (2011) and 2 (2012)

from the partially excluded carcasses (Fig. 18.5a), however there were no significant differences between any of the measurements ($p > 0.001$).

The pH values in Trial 2 displayed more fluctuations than observed in Trial 1 and there was the addition of the completely excluded carcass. The control and completely excluded carcasses yielded fluids with pH values around 7.5 initially, while the partially excluded carcasses displayed pH values between 8.5 and 9 (Fig. 18.5b). All pH values decreased below 7 on day 6 (101 ADD) which coincided with the late bloat stage for the controls and the mid bloat stage for the experimental groups. The pH values then increased to values between 7 and 7.5 until day 9 (157 ADD) which

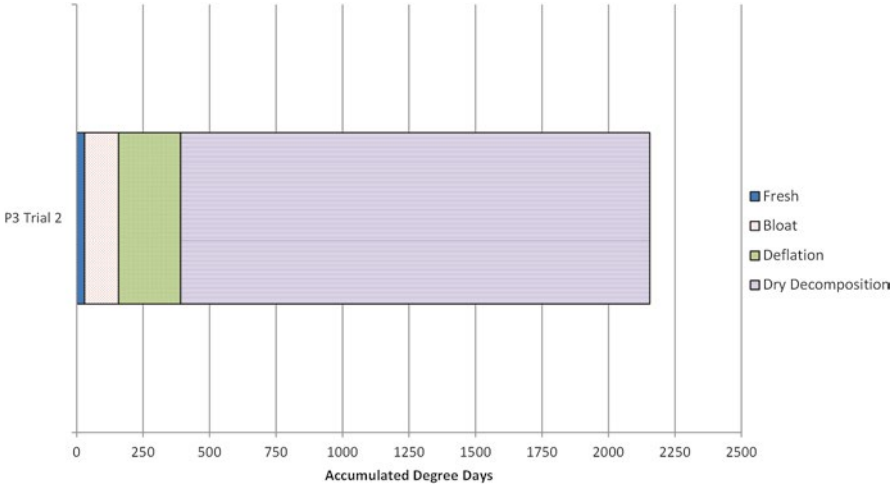

Fig. 18.4 Onset of the decomposition stages for the completely excluded carcass in Trial 2 (2012)

coincided with the beginning of the active decay stage for the controls and the end of the bloat stage for the experimental group. After this point, the trends and pH values diverged between carcass groups. The values increased for the inclusion and partially excluded groups, with the inclusion group having more alkaline values than the partial exclusion group. These values subsequently decreased after day 13 (238 ADD; beginning of advanced decay for the inclusion carcasses; during localized tissue removal for the partially excluded carcasses) until the end of the study, with final values recorded between 7.5 and 8. After day 9 (157 ADD; end of bloat), the completely excluded carcass decreased in pH until day 15 (275 ADD; mid deflation), after which point it increased and followed similar trends as the partially excluded group, with final values between 7.5 and 8. There were no significant differences between the inclusion and partial exclusion groups ($p > 0.001$). Statistics could not be performed on the completely excluded carcass because there were no replicates, so it is undetermined whether there were significant differences between the completely excluded group and the other groups.

18.3.4 Conductivity Measurements

At the beginning of Trial 1, the conductivity measurements of each group followed very similar trends (Fig. 18.6a). The lowest values were recorded on day 3 (62 ADD; early bloat) (Fig. 18.6a). The values then steadily increased until day 8 (169 ADD; end of active decay for the inclusion carcasses and early localized tissue removal for the partially excluded carcasses), at which point the values decreased until day 13 (256 ADD; early advanced decay for the inclusion carcasses and end of localized tissue removal for the partially excluded carcasses). The partial exclusion group then demonstrated another cycle of increasing and decreasing conductivity values until the end of the study. The control group exhibited similar trends

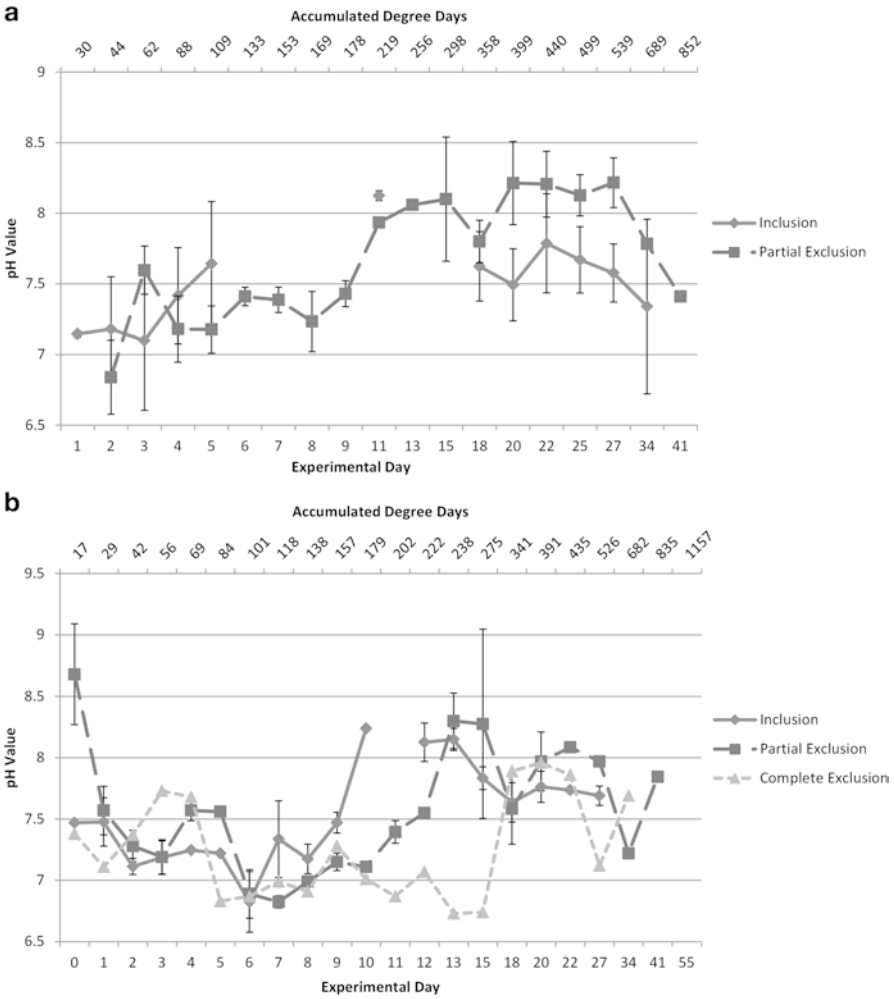

Fig. 18.5 (**a**) pH values of decomposition fluid collected from inclusion and partial exclusion carcasses during Trial 1 (2011); (**b**) pH values of decomposition fluid collected from inclusion, partial exclusion, and complete exclusion carcasses during Trial 2 (2012)

following day 18 (358 ADD; early advanced decay), however the fluctuation in values was less evident. Fluid samples from the controls were not collected between days 6 and 9 (133–178 ADD; active decay) and 13–15 (256–298 ADD; early advanced decay) and it is therefore unclear whether they followed the same trends as the partial exclusion group during that time. There were no significant differences between any of the measurements ($p > 0.001$).

The conductivity measurements of each carcass group in Trial 2 also displayed similar values and trends during early decomposition (Fig. 18.6b). The values were initially low, with the lowest values recorded on days 2 and 3 (42 and 56 ADD; beginning of bloat). The values of all groups showed increasing trends until day 9

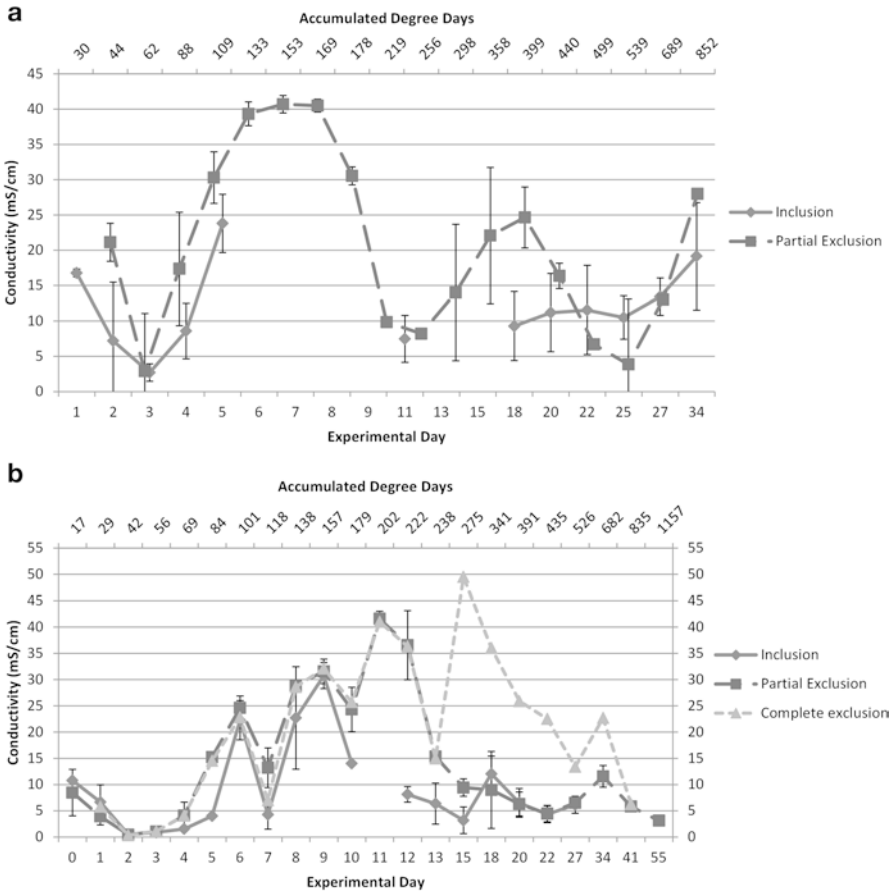

Fig. 18.6 (**a**) Conductivity measurements of decomposition fluid collected from inclusion and partial exclusion carcasses during Trial 1 (2011); (**b**) Conductivity measurements of decomposition fluid collected from inclusion, partial exclusion, and complete exclusion carcasses during Trial 2 (2012)

(157 ADD; beginning of active decay for the controls, and end of bloat for the experimental groups), with decreased values on day 7 (118 ADD; late bloat) and day 10 (179 ADD; early active decay for the inclusion carcasses, beginning of localized tissue removal for the partially excluded carcasses, and beginning of deflation for the completely excluded carcass). After day 9 (157 ADD), the trends and conductivity measurements from each carcass group diverged. The values from the inclusion group decreased and remained low, while the exclusion groups remained high. The partial exclusion group subsequently decreased and remained low, while the decrease in values from the complete exclusion carcass was delayed, but remained higher in value until the last measurement. There were no significant differences between any of the values for the inclusion and partial exclusion groups ($p > 0.001$). Statistics could not be performed using the completely excluded group because of a lack of replicates.

Table 18.4 The major infrared stretching bands detected in decomposition fluid

Wave number (cm^{-1})	Assignment
3150–3000	Unsaturated fatty acid = C–H stretching
2950–2800	C–H stretching
1730–1700	Saturated fatty acid C=O stretching
1680–1620	Unsaturated fatty acid C=C stretching
1576–1540	Salts of fatty acids

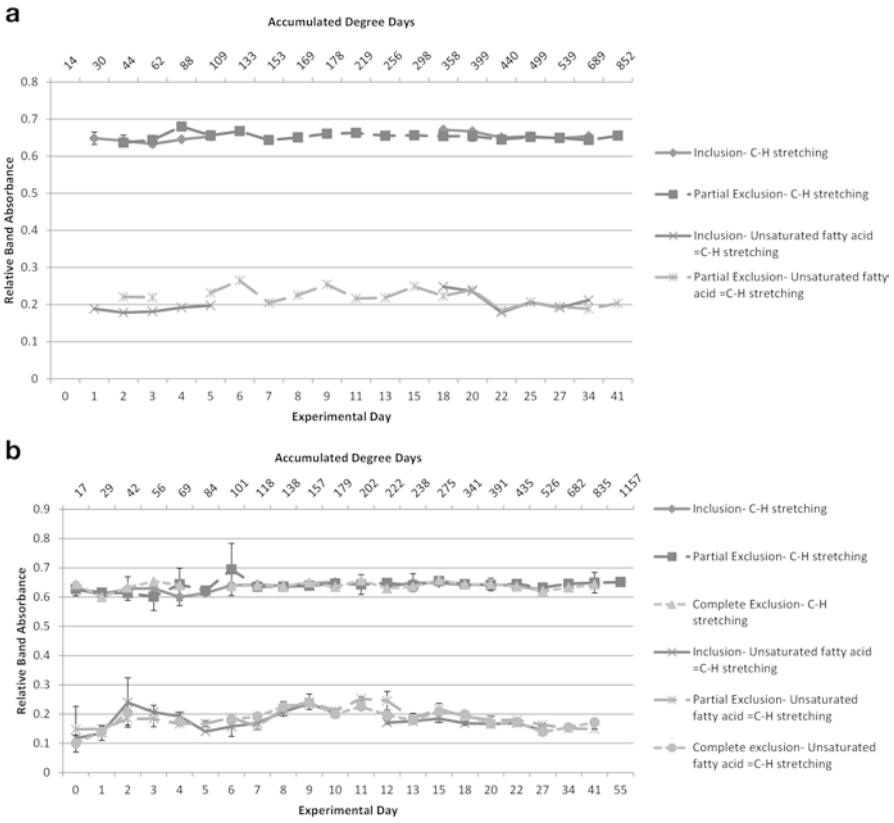

Fig. 18.7 ATR-IR spectroscopy results for early C–H stretching and unsaturated fatty acid =C–H stretching bands from: (**a**) Trial 1 (2011) and (**b**) Trial 2 (2012)

18.3.5 ATR-IR Spectroscopy

Several infrared stretching bands were consistently observed in all samples and are listed in Table 18.4. Stretching bands between 3150 and 3000 cm^{-1} were consistently low, while those between 2950 and 2800 cm^{-1} were consistently high (Fig. 18.7). Neither bands fluctuated noticeably over the course of Trial 1 or 2. The absorbance of the saturated fatty acid C=O stretching bands (1730–1700 cm^{-1}) was typically higher than the absorbance resulting from the unsaturated fatty acid C=C stretching bands (1680–1620 cm^{-1}) in both Trials 1 and 2 (Fig. 18.8).

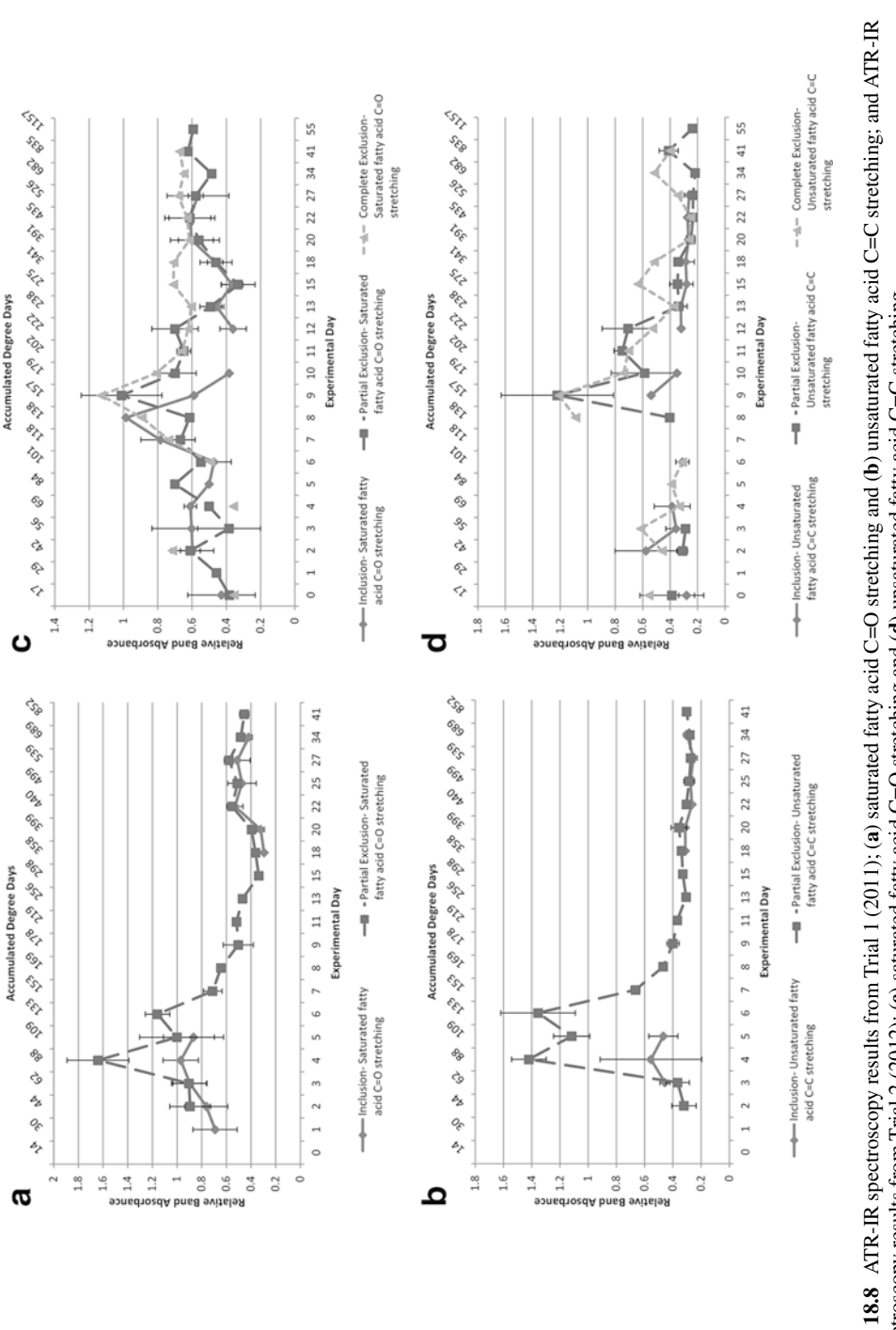

Fig. 18.8 ATR-IR spectroscopy results from Trial 1 (2011); (**a**) saturated fatty acid C=O stretching and (**b**) unsaturated fatty acid C=C stretching; and ATR-IR spectroscopy results from Trial 2 (2012): (**c**) saturated fatty acid C=O stretching and (**d**) unsaturated fatty acid C=C stretching

The saturated fatty acid stretching bands between 1730 and 1700 cm^{-1} and the unsaturated fatty acid stretching bands between 1680 and 1620 cm^{-1} displayed similar trends during Trial 1 (Fig. 18.8a and b). Sharp increases in levels in the partial exclusion group occurred after day 4 (88 ADD; mid bloat), which subsequently decreased and shared similar values with the inclusion group after day 18 (358 ADD; early advanced decay for the inclusion group and early dry decomposition for the partially excluded group) (Fig. 18.8a and b). The levels of saturated fatty acids decreased to values that were below the initial absorbance, while the levels of unsaturated fatty acids were similar to the initial levels following the increase during early decomposition (Fig. 18.8a and b).

In Trial 2, the absorbance values from all bands appeared to fluctuate more than in Trial 1. All carcass groups shared similar absorbance values at the beginning of the study for both the saturated C=O (1730–1700 cm^{-1}) and unsaturated C=C (1680–1620 cm^{-1}) fatty acid stretching bands (Fig. 18.8c and d). Sharp increases in the levels of saturated fatty acids were displayed by all groups around days 8–9 (138–157 ADD; beginning of active decay for the control group and end of bloat for the experimental groups) and in the exclusion groups for the unsaturated fatty acids (Fig. 18.8c and d). The sharp increase in unsaturated fatty acids among the exclusion groups was followed by a gradual decrease until the end of the collection period (Fig. 18.8d). The values for saturated fatty acids were higher at the end of the study, compared to initial values, while the unsaturated fatty acids were slightly lower (Fig. 18.8c and d).

The absorbance values for saturated and unsaturated fatty acids displayed by the inclusion groups were always lower than the other groups (Fig. 18.8). The completely excluded carcass typically exhibited the highest absorbance values for these free fatty acids (Fig. 18.8c and d).

The bands indicative of fatty acid salts (1576–1540 cm^{-1}) from the partial exclusion group showed a sharp increase early in the study during Trial 1, similar to the saturated and unsaturated fatty acid stretching bands, but fluctuated over time (Fig. 18.9a). The absorbance levels from the inclusion group were initially lower than the partial exclusion group, but reached similar values at the end of the study (Fig. 18.9a). During Trial 2, similar values were observed among all carcass groups following day 6 (101 ADD; end of bloat for the control group and mid bloat for the experimental groups) (Fig. 18.9b). Prior to that time, the complete exclusion carcass exhibited sharp increases (Fig. 18.9b).

18.4 Discussion

Fluids collected from all carcasses during both trials initially had pH values close to 7. The fluid collected was predominantly blood which, in a living individual, should have a pH between 7.35 and 7.45 (Waugh and Grant 2010). When the pH of blood is outside this narrow range, it indicates a disruption to the normal physiological and biochemical processes (Waugh and Grant 2010). Death leads to the cessation of regulatory mechanisms within the body (Janaway et al. 2009) and as a result, the pH of cadaver blood will be slightly outside the normal range.

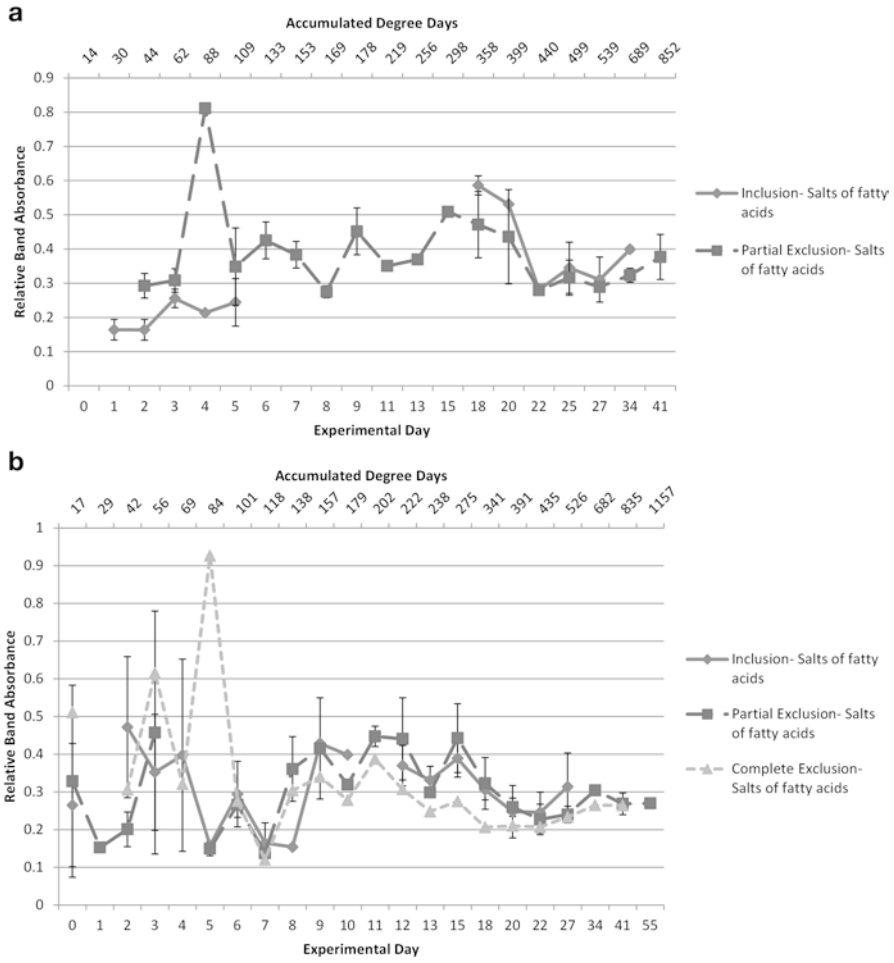

Fig. 18.9 ATR-IR spectroscopy results for salts of fatty acids from **a** Trial 1 (2011) and **b** Trial 2 (2012)

The pH values of collected fluid displayed similar trends and values between groups, based on the available data for the inclusion group in Trial 1 and during early decomposition in Trial 2. The pH values began to increase in all carcass groups near the end of the bloat stage. This may have been caused by the onset of proteolysis, which has been observed to lead to an increase in intracellular pH levels (Gill-King 1997). However, once the active decay and localized tissue removal stages began, the pH values diverged between groups. This was especially evident following experimental day 9 (157 ADD) in Trial 2. The fluid from the inclusion group exhibited higher pH values than the partial and complete exclusion groups and displayed a sharp increase in pH. Studies have found that high ammonium (NH_4^+) levels contribute to high pH values (Hopkins et al. 2000). Maggots release high levels of ammonia in their exudates as they feed (Turner 2005), which can be converted to ammonium and subsequently increase pH. The partially excluded group displayed

an increase in pH following day 10 (179 ADD), which correlated with the beginning of the localized tissue removal stage, when maggots were present to contribute ammonium. Fewer maggots were present on these carcasses which may explain why the pH increased at a later ADD relative to the inclusion group. The completely excluded carcass displayed a decrease in pH, which may be due to the lack of maggots and ammonium ions present in that environment.

During late decomposition, the pH values for the inclusion and partially excluded groups began to decrease, while the values for the completely excluded carcass increased to reach similar values as the partially excluded group. The inclusion values were always lower than those from the partially excluded groups during this time. It is possible that the nature of the remains contributed to the differences observed between the inclusion and the exclusion (partial and complete) groups. During late decomposition, only bones and skin remained among the inclusion carcasses, while large amounts of soft tissue were still present among the partially and completely excluded groups, due to the limited or lack of feeding by maggots. Microbial-driven decomposition was still taking place among the partial and complete exclusion carcasses during the later stages of decomposition (as evidenced by changes in soft tissue and fluid) and these changes may have maintained higher levels of pH among these groups. During the later decomposition stages, it was unclear whether the inclusion carcasses were indeed purging fluids or precipitation was causing fluids to accumulate in the containers. The latter may explain the lower pH values observed, since rainwater is considered to be relatively acidic and has been found to have a pH between 4 and 7 (Ferguson and Jeffries 2012). A study conducted in north-eastern United States found that the average annual precipitation had pH values ranging from 4.05 to 4.3 (Likens et al. 1996). Therefore, if the collected fluid was predominantly precipitation from the inclusion carcasses, it follows that the pH values would be lower than those from the partial and complete exclusion groups.

The conductivity values and trends exhibited by the carcass groups within each trial were very similar during early decomposition. All groups displayed a general increase in conductivity during the fresh and bloat stages. Vass et al. (1992) showed that several ions (mainly NH_4^+, K^+, Cl^-, and SO_4^{2-}) increase sharply during early decomposition and continue to increase over time. These ions would have been released by all carcasses and likely influenced the increase in conductivity observed. All carcass groups also displayed noticeable decreases on the same days within each trial. In Trial 1, this occurred on experimental day 3 (62 ADD) and in Trial 2, on days 2 (42 ADD), 3 (56 ADD), 6 (101 ADD), 9 (157 ADD), and 12 (222 ADD). These represent days that either received, or followed days that received, large amounts of precipitation. Water dilutes fluids and will dilute the amount of total dissolved ions present in a given fluid, thus decreasing its conductivity. A significant decrease in conductivity was observed in a study that measured the conductivity in soil solution collected from a burial site and it was attributed to the increased rainfall received during that time (Pringle et al. 2010).

Differences in conductivity were observed between groups following the active decay and localized tissue removal stages in Trial 2. The conductivity of the inclu-

sion carcasses decreased after this point and remained low, while the conductivity of the partial and complete exclusion groups remained high and continued to increase. The partially excluded group decreased a few days after and remained low, while the completely excluded carcass remained higher in value than the other two groups and gradually declined until the end of the study. This difference between groups must have been caused by the presence of maggots. Electrolytes rapidly leach out of soft tissue promoting an increase in conductivity, however bacteria have been shown to use many ions for metabolism (Vass et al. 1992) which would lead to a subsequent decrease in conductivity. It is possible that the maggots contributed bacteria or allowed the already present bacteria to access the released ions (mainly HPO_4^{2-}, HCO_3^-, and NO_3), thus leading to the decrease in conductivity in the inclusion and partially excluded carcasses. The delayed decrease observed in the complete exclusion carcass may have been due to fewer ionic compounds being released from the decaying carcass as time progressed, which can also explain why the values for the other groups remained low even after maggots had migrated away from the carcasses.

Strong –C–H stretching bands in the region 2950–2800 cm^{-1} and a small shoulder in the region 3150–3000 cm^{-1}, attributable to=C–H stretching of unsaturated fatty acids, were consistently observed and the absorbance did not fluctuate over time. These bands are commonly observed in infrared spectra resulting from fatty acid analysis of pork tissue or soil collected from beneath decomposing pigs (Flatten et al. 2005; Forbes et al. 2005a, b, 2011).

The fatty acid absorbance levels exhibited by the inclusion group in both trials were typically lower than the other groups, however they appeared to reach similar levels during the later stages of decomposition. Further, a divergence in trends between groups was observed for the saturated fatty acid C=O stretching band after day 8 (138 ADD) in Trial 2, which was the onset of the active decay stage. The inclusion group decreased sharply and remained low. The feeding of maggots removed soft tissue and liquefied tissues that contained fatty acids, thus decreasing the levels released into fluid. This resulted in lower levels being detected in the purged fluids. Similarly, a decrease in absorbance was also exhibited by the partial exclusion group at the beginning of the localized tissue removal stage. However, the completely excluded carcass decreased at the same time (for both the saturated fatty acid C=O stretching and the unsaturated fatty acid C=C stretching). This indicates that maggots are not the only factor involved in the decrease of saturated fatty acid levels. It is likely that fatty acids are also chemically degraded, however insects may accelerate the process and lead to increased lipid degradation.

The absorbance levels of all groups appear to reach similar values near the end of the collection period, despite different degradation pathways during earlier stages. This was especially true in the saturated fatty acid C=O stretching band in Trial 2, whereby the inclusion and partial exclusion group levels increased to reach the same values as the completely excluded carcass. A slight increase was also observed at the end of the collection period in Trial 1. The unsaturated fatty acid C=C stretching band displayed a decreasing trend in all carcass groups near the end of the study. This results not only from the natural degradation processes but also as

a result of hydrogenation, which converts unsaturated fatty acids into their saturated counterparts (Dent et al. 2004; Janaway et al. 2009; Notter et al. 2009). This process may explain the slight increase in absorbance of the saturated fatty acid C=O stretching bands and the low levels of the unsaturated fatty acid C=C stretching bands during the later stages of decomposition.

A study conducted by Swann et al. (2010b) investigated long chain fatty acids in decomposition fluid collected from adult pig carcasses. They observed that the levels of fatty acids displayed an increasing trend over time and reached a maximum after 14 days (310 ADD) (Swann et al. 2010b). Unfortunately, the study was terminated at this time, so further trends could not be observed. The increase in fatty acid content (exhibited by the saturated fatty acid C=O and unsaturated fatty acid C=C stretching bands) peaked after 4 days (88 ADD) in Trial 1 and after 8–9 days (138–157 ADD) in Trial 2, which is much earlier than observed by Swann et al. (2010b). The authors explained a cycle in fatty acid content observed in fluid collected from pork rashers as being caused by the level of fly activity, the feeding cycle of maggots, and the adipose tissue content of the carcass (Swann et al. 2010b). It was hypothesised that as maggots fed on tissues, the production of long chain fatty acids steadily increased, and once they migrated from the carcasses to pupate the levels decreased (Swann et al. 2010b). This is contrary to what was observed in the current study, whereby in Trial 2, the peak in fatty acid content correlated with the onset of the maggot feeding stages and decreased throughout this stage and subsequent stages. Similar trends were also observed by the completely excluded carcass, which was not exposed to any insect activity.

The bands indicative of salts of fatty acids exhibited absorbance levels that were similar between all groups. However, the completely excluded carcass displayed higher levels during the early stages of decomposition. The fresh and bloat stages took place during this time, which are stages exhibited by all carcasses. Similar chemical changes would therefore have taken place within each carcass. Further, maggots were not present during this stage and the differences between groups cannot be attributed to insect activity. It is unclear as to why the completely excluded carcass displayed higher fatty acid salt levels compared to the other groups, especially since all carcass groups displayed similar levels following day 9 (157 ADD) in Trial 2, which was when the absorbance levels typically diverged between groups. It can be concluded that the fatty acid salt levels are not influenced by insect activity.

18.5 Conclusions

Few studies have examined the decomposition process in both the presence and absence of insects, and to the best of our knowledge, only one study has performed fatty acid analysis using decomposition fluid. The current study combined these two knowledge gaps in an attempt to determine the effect of insect activity on the chemical properties of decomposition fluid. It was observed that the pH and conductivity of fluid appear to be influenced by maggot activity. The feeding of maggots caused

increases in pH among the inclusion and partial exclusion groups, although the partial exclusion group displayed a more gradual increase. The presence of maggots likely led to higher levels of ammonium, thus increasing the alkalinity of the fluid. In contrast, the presence of maggots led to decreases in conductivity. It is hypothesized that bacteria from maggots utilised the electrolytes that were released by the degradation of macromolecules, thereby decreasing the levels of dissolved ionic compounds. There were differences observed in the absorbance levels of fatty acids detected using ATR-IR, however the overall levels were not sufficiently different between carcass groups to accurately determine whether the presence of insects played an important role in the fatty acid degradation process. Qualitative and quantitative methods are currently being performed using gas chromatography-mass spectrometry (GC-MS), in an attempt to better identify specific fatty acid trends in decomposition fluid.

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