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Insect Succession on Wrapped Rabbit Carcasses as a Model in Forensic Entomology

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ABSTRACT

One of the roles of insects in life is as an agent in nutrient cycling together with microorganisms, through decay and degradation of matter. Applications in that role are utilized for the development of forensic entomology, which can help solve the problem of timing and causes of death. A person who dies because of crime is often found in a wrapped condition which interferes the decomposition process and makes it difficult to identify the timing of the incident. This study aims to determine the effect of wrapping on the decay process associated with the presence of insects using rabbits as animal models. The research was conducted in Purwoasri village, Metro Utara sub-district, Lampung province, using 3 male rabbits weighing 2-3 kg and quickly killed by cutting the carotid artery. Carcass I was wrapped in a sack, carcass II as control was not wrapped, and carcass III was wrapped in a plastic. Observations included the microclimate measurements, the carcasses temperature and process of decay, and the presence of species and numbers of insects. The results showed that the microclimate environment where each carcass was placed was not significantly different, although in the first 24h, the carcass temperature of both wrapped rabbits was higher than the ambient temperature. All rabbits undergo a process of decomposition starting from the fresh stage, bloated stage, decay stage, post decay stage, and skeletal stage, but at varying speeds. Post decay in carcass II (control), in carcass I (sack), and carcass III (plastic), occurred at 72h, 120h, and 192h postmortem, respectively. The skeletal stage for carcass II (control) entered at 96h while for carcass I (sack) entered at 144h postmortem. The skeletal stage for carcass III (plastic), however, occurred at more than 220h. Diptera were present at the beginning of fresh stage, while Coleoptera was starting present at the decay stage. Diptera of *Musca domestica* and *Chrysomya albiceps* and Coleoptera of Histeridae were the most dominant with varying numbers in each carcass. From this study, it can be concluded that wrapping affected the temperature of carcasses, and subsequently affected the decay process, also the number and the species of the insect presents.

***Key words**: Cylindroiulus sp., Decomposing agent, Morphological characters, Solid waste, Surabaya*

Introduction

Forensic entomology is a branch of science that studies applications of arthropods such as insects for legal purposes (Catts and Goff, 1992). The arrival and departure of insects on a corpse produces predictable patterns related to the decomposition phase of the corpse. These patterns became known as insect succession (Gennard, 2007). Insect groups that have an important function in forensic entomology studies are the order of Diptera and Coleoptera. Both orders can detect the presence of a corpse in a short time after death and are the main types of insects that visit the corpse (Gennard, 2007).

In Indonesia, the use of insects in forensics is not yet popular because it is constrained by various environmental factors that affect the decay process, such as the temperature and the humidity. In connection with the many discoveries of bodies in cases of wrapped crimes, it is necessary to conduct research on insect succession from wrapped carcasses. It can provide an overview of the insect succession patterns in wrapped carcasses.

The research was conducted using rabbits as animal models, each of which was wrapped in a plastic and in

Materials and Methodology

The research was conduct in Purwoasri village, Metro Utara sub-district, Lampung province, Indonesia, using 3 male rabbits weighing 2-3 kg, which were killed by cutting the carotid artery in the neck (ethics commission permit, Institut Teknologi Bandung). The first carcass was wrapped in a sack, the second was left without wrap, and the third was wrapped in a plastic. Each carcass was put in a cage to avoid interference by other animals, then placed under trees with distance of 30 meters.

Microclimate measurements, namely temperature, light intensity, and humidity, were carried out using a sling psychrometer and lux meter twice a day, before and after observing the carcass. The process of decay was observed while the temperature of carcass was measured using a thermometer. All types and stages (egg, larvae, pupae, adult) of insects were collected daily using a sweeping net and tweezers, then put them immediately into bottles containing 70% of alcohol. Insects identification were carried out using a Nikon SMZ445 stereo microscope and identification keys from Amendt in 2000 (Amendt, 2000).

Results and Discussion

The results of the microclimates in research area are shown in Table 1. As it shows in Table 1, the measured microclimates, namely temperature, light intensity, and humidity, were not significantly different at the three locations where the carcasses were placed, as the P-value by using ANOVA Test were higher than 0.5. Based on these results, what will differentiate the carcasses' microclimate is the wrapping treatment (sack or plastic) compared to the unwrapped.

Data about carcass temperature shows on Figure 1 and 2. Figure 1 shows that during first 24 hours, the average temperature of carcass I (sack) was between 26-31 ^{o}C, while the average temperature of the environment was between 27.4-31.75 ^{o}C. Statistical t-test shows a significance value (2-tailed) of 0.738 (sig > 0.05), which means that there is no significant difference between the two.

Figure 2. shows the average temperature of carcass III (plastic), which was between 29-34 ^{o}C while

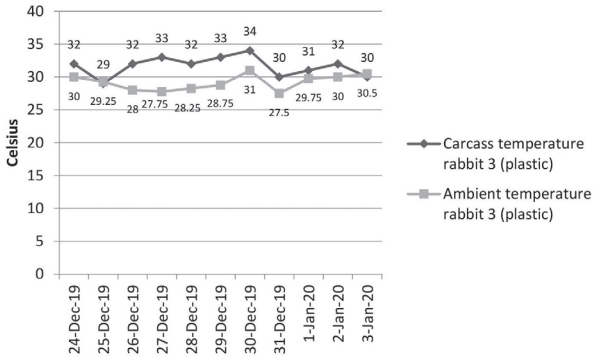

Fig. 1. Carcass I (sack) temperature compared to ambient temperature

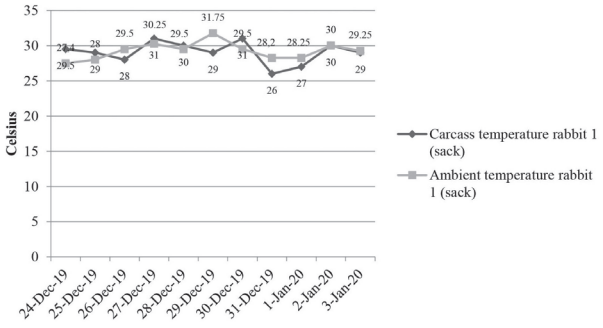

Fig. 2. Carcass III (plastic) temperature compared to ambient temperature

Table 1. The average of microclimate measurement in 3 locations where carcasses were placed

	Location I	Location II	Location III	ANOVA Test
Average Temperature	28.25- 31.75^{o}C	28.25- 31.75^{o}C	27.50 – 31.00^{o}C	(P-value) = 0.789
Average Light Intensity	4587.5-15293.75 lux	4700- 19475 lux	5028.75- 19227.5 lux	P-value = 0.810
Average Humidity	67.75-87.50%	69.25-87.00%	69.50-86.50 %	(P-value) = 0.608

the average temperature of the environment was between 28-31 °C. Statistical t-test shows a significant value of 0.000 (sig < 0.05), which means that there is a significant difference between the two.

No significant differences in carcass I (sack) was most probably because the sack have small holes, so that the air still moving in and out, while significant differences in carcass II (plastic), was affected by the plastic cover itself since warm temperature produced from decaying process was trapped. The process of declining body temperature until it reaches environmental temperature is called rigor mortis which can happen through 4 mechanisms, namely radiation, convection, conduction, and evaporation (Myburgh, 2010; Hau, 2014). The process of declining carcass temperature until it reaches environmental temperature happened on 24 h post-mortem (Clark and Worrell, 1997). As shown on figure 1 and 2, carcass I and carcass II experienced a decrease in carcass temperature to be the same as the ambient temperature on

December 25 2019 (24 hours post mortem).

Four decomposition stages were observed, namely fresh, bloating, decay, post decay, and skeletal remain in this research, as it shows in Table 2. Carcass I (sack) and carcass III (plastic) underwent a decomposition stage until skeletal remain for 144 hours and more than 240 hours after death, respectively. Compared with the carcass II (control), which lasted for 96 hours, the process in wrapped carcasses lasted longer.

Insects, apart from microorganisms, play an important role in the process of putrefaction. When access to the carcass is blocked, the decomposition process is also delayed. According to Moffatt in 2017, the longer decomposition process of carcass wrapped in plastic is largely due to the smaller amount number of insects in the plastic. This causes the amount of oxygen to be lower and form an anaerobic environment which slows down the rate of decomposition (Widya, 2012).

The insects that come to the carcass have a sequence according to the stage of decomposition that occurs. Each stage of decomposition attracts different insect species. The *Diptera* species found at each decomposition stage in this study are shown in Table 3.

In the fresh stage, the family Muscidae (*Musca domestica, M. vetustissima, M. sorbens*), Calliphoridae (*Chrysomya rufifacies, Lucilia caesar, L. sericata*) and Sarcophagidae (*Sarcophaga carnaria*) were found. This result is similar with Supriyono's research in 2019, that the presence of adult insects in the early stages of death is dominated by fly insects belonging to the order of Diptera (Calliphoridae, Muscidae, and Sarcophagidae).

M. vetustissima and *C. albiceps* were found in carcass I (sack) and carcass II (control) when bloated stage occurred. *Musca vetustissima* is known to be attracted by the smell of ammonia sulfate and indole (Mulla, 1985). According to Vass in 2008, ammonia and indole are gases produced in the putrefaction process by microbes during bloated stage. The gas attracts insects such as *S. carnaria* (Nurokhman, 2018). *Sarcophaga carnaria* was observed in carcass III when bloated stage occurred.

In the decayed stage, there were more Diptera insects that play a role in decomposition compared to other stages. This is due to the opening of the body area of the carcass after the end of bloating stage.

In the dry or skeletal remains stage, there were fewer Diptera types on the carcass compared to the previous stage. This happens because the carcass as food has run out and Diptera only needs to com-

Table 2. Time the decomposition stage

Decomposition stage	Date										
	24-Dec-19	25-Dec-19	26-Dec-19	27-Dec-19	28-Dec-19	29-Dec-19	30-Dec-19	31-Dec-20	01-Jan-20	02-Jan-20	03-Jan-20
	0 h	24 h	48 h	72 h	96 h	120 h	144 h	168 h	192 h	216 h	240 h
Fresh	CI, CII, CIII	CI,CIII									
Bloated		CII	CI,CIII	CIII							
Decay			CII	CI	CI, CIII	CIII	CIII	CIII			
Post Decay				CII		CI			CIII	CIII	CIII
Dry					**CII**		**CI**				

plete the stages of their life.

The *Coleoptera* species observed in each phase of decay are shown in Table 4. In the decay stages, *Saprinus aeneus* and Hister congener were seen on carcass I (sack), and only *S. aeneus* on carcass II (control), while in carcass III (plastic) showed the presence of *Dermestes laniarius*, *Psilopyga nigripennis, S. aeneus*, and *H. congener*. According to Ozdemir and Sert in 2009; H. congener is one of the *Coleoptera* species from the Histeridae which is the second largest family found in carcasses and is usually in the decay stage.

In the dry or skeletal remain stage, *Tomogenius latipes* was observed in carcass I (sack) and carcass II (control). *Gnathusa alfacaribou* was found in carcass I (sack) and in carcass III (plastic). This insect is a species of the Staphylinidae and is a predator of larvae and other small animals on carcasses, commonly found in the decay and skeletal remain stages (Dekeirsschieter, 2013). On carcass I (sack) was found *Onthophagus gazella* from Scarabaeidae. According to Blume in 1975, *O. gazella* played a role in reducing the population of *M. vetustissima*, which was also found in this study. On carcass III (plastic), *Psilopyga nigripennis* from the Nitidulidae family were found. Nitidulidae are known as an important family that colonizes carcasses in the final stages of decomposition along with species from the Dermestidae family (Ortloff, 2014).

Number of the Diptera and Coleoptera samples in each treatment and in each stage of decay is shown in Figure 3. In carcass III (plastic), number of the Diptera was less than that of other carcasses, while number of Coleoptera was higher than in other carcasses (Figure 3. A). The plastic wrap blocks the access of Diptera to the carcass and blocks the decomposed gas, which plays a role in attracting Diptera, to escape. The Diptera eggs and larvae

Table 3. Diptera species found in each decomposition stage

Decomposition Stage	Diptera species found		
	Carcass I (Sack)	Carcass II (Control)	Carcass III (Plastic)
Fresh	• *Musca domestica* (Muscidae) • *Sarcophaga carnaria* (Sarcophagidae) • *Chrysomya rufifacies* (Calliphoridae) • *Musca vetustissima* (Muscidae) • *Musca sorbens* (Muscidae) • *Lucilia caesar* (Calliphoridae)	• *Musca domestica* (Muscidae) • *Sarcophaga carnaria* • *Musca domestica* (Muscidae) • *Sarcophaga carnaria* • *Lucilia sericata* (Calliphoridae)	
Bloated	• *Musca domestica* (Muscidae) • *Chrysomya rufifacies* (Calliphoridae) • *Musca vetustissima* • *Musca sorbens* • *Chrysomya albiceps* (Calliphoridae)	• *Musca domestica* (Muscidae) • *Musca vetustissima* • *Lucilia cuprina* (Calliphoridae) • *Lucilia caesar* (Calliphoridae) • *Chrysomya albiceps*	*Sarcophaga carnaria*
Decayed	• *Musca domestica* • *Chrysomya megacephala* (Calliphoridae) • *Chrysomya rufifacies* (Calliphoridae) • *Chrysomya albiceps* (Calliphoridae) • *Lucilia sericata* (Calliphoridae) • *Liopiophila varipes* (Piophilidae) • *Atherigona orientalis* (Muscidae) • *Minettia longipennis (Lauxaniidae)* • *Lucilia illustris* (Calliphoridae) • *Anthomyia ilocata*	• *Musca domestica* • *Chrysomya rufifacies* (Calliphoridae) • *Musca vetustissima* • *Chrysomya albiceps* • *Chrysomya megacephala* (Calliphoridae) • *Chrysomya putoria* • *Liopiophila varipes* • *Atherigona orientalis* • *Lucilia ilustris* (Calliphoridae) • *Lucilia eximia* (Calliphoridae) • *Chrysomya rufifacies* (Calliphoridae)	• *Musca domestica* (Calliphoridae) • *Chrysomya albiceps* (Calliphoridae) • *Liopiophila varipes* • *Atherigona orientalis* • *Minettia longipennis* • *Liopiophila varipes*
Dry (skeletal	• *Musca domestica* (Muscidae) • *Chrysomya megacephala* • *Chrysomya albiceps*	• *Musca domestica* (Muscidae) • *Liopiophila varipes* • *Anthomyia ilocata*	

were then located in open areas and on the outer and lower sides of the plastic that attract the Coleoptera. This result is also in accordance with research conducted by Mashaly in 2019, which shows that carcasses with cloth covers attract high number of Coleoptera and the otherwise of Diptera.

As shown in Figure 3 B, Diptera has arrived at fresh stage in all carcasses. The number increases with the changing stages of decay and reaches a peak in the decay stage. From the same figure, it also shows the pattern of insect succession on carcass, the first to arrive during the fresh stage is Diptera, followed by Coleoptera in the decay stage to skeletal remain. There are no significant different between wrapped carcasses and unwrapped carcass (control) in this study.

The distribution of numbers per species of Diptera and Coleoptera in each carcass is shown in Figure 4 and 5. The highest number of individuals Diptera in each carcass was *M. domestica* followed by *C. albiceps*. According to Supriyono in 2019, the two insects are the main colony types in the process of carcass decay.

The high number of *Musca domestica* in this study is probably due to the location of the research location which is not far from residential areas.

Residential areas are an environment where house flies can be found in large numbers (Koesharto *et al.*, 1986). The genus Lucilia is represented by five species, all of which are morphologically and ecologically similar, and can be found in exposed carcasses (Kuusela and Hanski, 1982). Because the Lucilia genus prefers open carcass conditions, the species of this genus are not found abun-

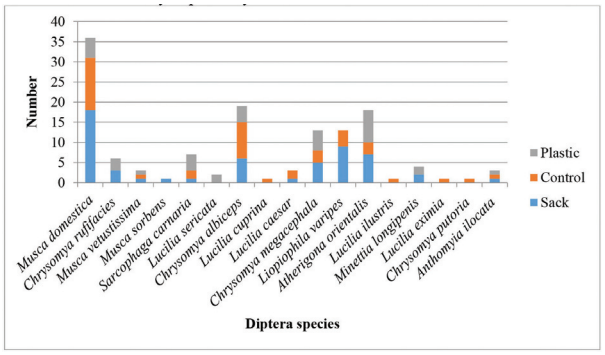

Fig. 4. Distribution of Diptera Insect Samples

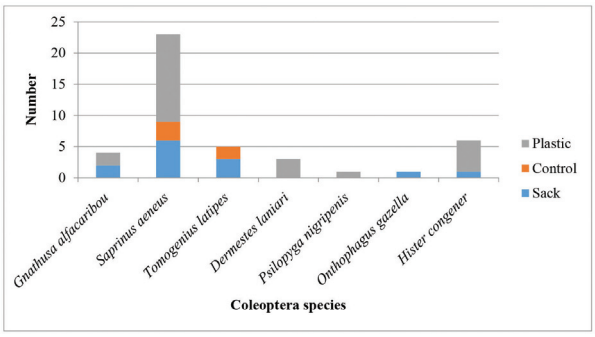

Fig. 5. Distribution of Coleoptera Insect Samples

Table 4. Coleoptera species found in each decomposition stage

Decomposition Stage	Species of Coleoptera found		
	Carcass I (Sack)	Carcass II (Control)	Carcass III (Plastic)
Fresh	-	-	-
Bloated	-	-	-
Decayed	*Saprinus aeneus* (Histeridae) *Hister congener* (Histeridae)	*Saprinus aeneus* (Histeridae) *Psilopyga nigripenis* (Nitidulidae) *Saprinus aeneus* (Histeridae) *Hister congener* (Histeridae) *Gnathusa alfacaribou* (Staphylinidae)	*Dermestes laniarius* (Dermestidae)
Dry (skeletal remain)	*Onthophagus gazella* (Scarabaeidae) *Saprinus aeneus* (Histeridae) *Tomogenius latipes* *Gnathusa alfacaribou* (Staphylinidae)	*Tomogenius latipes* (Histeridae)	

dant in carcass I (sack) and carcass III (plastic).

S. aeneus, T. latipes, and *H. congener* dominated the number of samples in this study. The three species are *Coleoptera*, which belong to the family of Histeridae. *Gnathusa alfacaribou* is a species of the Staphylinidae family was also found to be quite abundant in this study.

Conclusion

Plastic wrap and sacks slow down the decomposition of rabbit carcasses. A carcass wrapped in plastic takes >240 hours, a carcass wrapped in sack takes 144 hours, while a carcass without a wrap takes 96 hours to reach the dry/skeletal remain stage. Plastic wrapping reduced the number of Diptera and increased the number of Coleoptera, while sack wrapping increased the number of Diptera and Coleoptera. The pattern of insect succession on all carcasses began with the arrival the Diptera of Calliphoridae, Muscidae, and Sarcopahgidae families, then Diptera of Piophilidae and Lauxaniidae families in the advanced decay stage, and continued with Coleoptera of Histeridae, Dermestidae, and Staphylinidae families in the decay stage to skeletal remain.

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