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Original Research

Influence of Different Types of Mutton Substrates on The Breeding Preferences of Necrophagous Flies

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Abstract

The present study was carried out to identify the breeding preferences of necrophagous flies to various types of mutton substrates i.e., fresh, cooked, salt, and turmeric powder treated and frozen forms. On day one of the experiment, the adult flies were recorded in group M1 and no flies were attracted to the remaining groups (M2 – M4). Fly eggs and first larval instars started to appear in the M1 group from the 2nd day onwards. By the 4th day onwards, adult flies, eggs as well larval instars were noticed in all the groups (M1 – M4). On the final day of the experiment, L3 stages were recorded with a varying number of all types of meat samples of mutton. In the present study, flies were attracted more immediately to fresh mutton meat than to remaining formulations. Other forms of mutton viz., cooked, salt, and turmeric treated and frozen samples were observed in the flies only after a day of its placement. The larvae obtained from fresh mutton (M1) samples were of Calliphora spp. (60%) and Lucilia spp. (40%). The majority of the dipteran larvae in cooked mutton (M2) were of Chrysomya spp. (73.3%) and the remaining (26.7%) were of Musca spp. In the third group of salt and turmeric powder treated mutton sample (M3), the obtained larvae were identified as Sarcophaga spp. (n=16) and the rest of the larvae were of Chrysomya spp. (n=14). The findings revealed that different Physico-chemical characteristics and also the proximate composition of mutton had significantly (P<0.05) influenced the breeding preferences of flies.

Keywords: Breeding preferences, Fly attraction, Mutton, Necrophagous flies, Processing

Introduction

Blow flies can cause the problem of myiasis in livestock and poultry. Flies, most commonly Calliphoridae, have frequently been associated with disease transmission in humans and animals, as well as myiasis. These larvae, commonly seen on decaying bodies, feed on carrion while the adults can be necrophagous or vegetative. During the process of decay, microorganisms such as *Mycobacterium* and *Salmonella* may be released through the body. Flies arrive at the scene and lay their eggs and the larvae begin eating the corpse, simultaneously ingesting the organisms. Although the strike is not limited to blow flies, these maggots are causing skin lesions, which, if severe enough, may be lethal. Strike starts when blow flies lay eggs in a wound or faecal material then followed by the development of maggots developed and they begin feeding tissues. With the ability to lay hundreds of eggs in a lifetime and the presence of thousands of larvae at a time in such close proximity, the potential for transmission is high, especially at ideal temperatures. The clinical signs depend on the tissue invaded, if it is in the skin, the infection starts as itchy sores then develops into painful boil-like lesions which often ooze.Most flesh flies breed in carrion, dung, or decaying material, but a few species lay their eggs in the open wounds of mammals. The pH of meat ranging from 7.13-8.23 is most suitable for larval development (Rasmussen and Campbell 1981) and the substrates that are adjusted within the moisture range promote the successful development of different flies. The presence of fat content and moisture also significantly affect the variability in development and oviposition preference by different species of flies (Schmidtmann 1992). Similarly, carrion breeding dipteran flies in the disintegration of chicken carcasses in Malaysia was also recorded by Abdulah *et al* (2015). Such kind of reports from India are lacking and initiation was made to take up this topic in the Chittoor district of Andhra Pradesh with the objective to explore the information to record the breeding preferences of necrophagous flies with different types of mutton meat substrates.

Materials and Methods

Meat Samples Collection and Categorization

The collected different chicken meat substrates were categorized into four groups i.e., Group-1: Fresh meat (Directly collected from meat shops and slaughterhouses); Group-2: Cooked meat (Cooked thoroughly at boiling temperature for about 15-20 minutes); Group-3: Salt and Turmeric powder treated meat (About 5 gm of salt and 3 gm of turmericpowder was mixed and kept for 2 hrs at room temperature); Group-4: Frozen meat (Stored at -20ºC for 24 hrs).

Determination of Meat Preferences by Necrophagous Flies

Exposure of Meat Samples to Flies

Fresh (n=4), cooked (n=4), salt and turmeric powder treated (n=4), and frozen meat samples (n=4) were categorized into Group 1 to Group 4, and in each group chicken (C), mutton (M), beef (B) and pork (P) were kept for the attraction of flies. Each type of meat sample was kept separately in clean plastic trays in an open area during the daytime. Care was taken to prevent access to the meat samples by the birds and other carnivores. Further, the exposed meat samples were maintained in a moist condition and protected from direct sunlight. These samples were monitored regularly with an interval of 3 hr and 8 hr during day hours and night hours, respectively up to 7 continuous days, such as the appearance of flies on the meat, laying of the eggs, development of larva, etc., time of first appearance of different flies on various meat samples, duration of egg laying and development of larvae were also recorded.

Collection and Processing of Fly Larvae

Fly larvae were observed (L_1, L_2 & L_3 instars) on the exposed meat samples and mature larvae (L_3) were collected for further processing and identification. The collected fly larvae were processed as per the description of Soulsby (1982) and AlanWalker (1994).

Identification of the Larvae

Morphological identification of fly larvae developed in the different types of meat samples was done by examination of the posterior spiracles of the respective larvae as per thedescriptions of Alan Walker (1994) and Soulsby (1982).

Evaluation of Physico-chemical Properties of Meat Sample

Physico-chemical properties of the meat samples used in the experiment were studied.pH was calculated as per the procedure outlined by Trout *et al* (1992) and water-holding capacity (WHC) was determined according to Wardlaw *et al* (1973). The water activity was calculated at a temperature of 25°C. The sample was filled in a dish/ cup to the brim to the maximum possible extent and the dish was kept in an airtight chamber taking care that the sample does not touchthe diaphragm of the sensor. The measuring chamber was conditioned to the temperature of the sample. The chamber was then closed in a manner to ensure metallic contact between the two parts. The values of water activity and the temperature were noted from the display panel when it reached the equilibrium and a constant reading is observed. 2-Thiobarbituric acid reactive substances (2-TBARS) value of different types of the chicken substrate was determined based on the procedure of Witte *et al* (1970). Free fatty acids (%) were determined according to the method described by Koniecko (1979). The proximate composition was calculated as per AOAC (2002).

Statistical Analysis

The generated data were subjected to analysis of variance, (one-way ANOVA for quality parameters). The smallest difference ($D_{5\%}$) for two means to be significantly different ($P<0.05$) was reported by Snedecor and Cochran (1995).

Results and Discussion

Observations of Flies/larval Stages on Meat Samples

The different mutton substrates samples [fresh meat, cooked meat, salt and turmeric powder treated (2 hrs) and frozen (-20^{O}C for 24 hrs)] were kept in a separate plastic tray in a common area with a distance of at least one foot between the meat samples. The meat samples were monitored for dipteran fly activity for a continuous period of 7 days with an interval of 3 hours and eight hours during the day and night hours, respectively. The details of the attracted flies and the larval stages were noted. On day one of the experiment, the adult flies were recorded in group M1 and no flies were attracted to the remaining groups (M2 – M4). Fly eggs and first larval instars started to appear in the M1 group from the 2nd day onwards. By the 4th day onwards, adult flies, eggs as well larval instars were noticed in all the groups (M1 – M4). On the final day of the experiment, L3 stages were recorded with a varying number of all types of meat samples of mutton (Table 1). In the present study, flies were attracted more immediately to fresh mutton meat than to remaining formulations. Other forms of mutton viz., cooked, salt, and turmeric treated and frozen samples were observed in the flies only after a day of its placement. The attributed reasons for this may be due to low water holding capacity in cooked meat and the common kitchen ingredients like salt and turmeric may be prevented to attract the flies. The drop in temperature of frozen meat may be a possible factor for the delay in attraction for the flies. Similar observations were found for meat samples from sheep,cattle, and pigs. Brannon (1934) observed the blowfly, *Lucilia sericata* breeding behaviour on ground beef, immediately after the placement of ground beef, the fly swarm over the meat almost immediately was noticed and begin feeding.

Table 1: Fly attraction and larval development stages observed on mutton

Day	Temperature (°C)	Relative Humidity (%)	Group			
			M1	M2	M3	M4
Day 1	32-37	68-70	F	F_0	F_0	F_0
Day 2	30-35	68-70	F, E, L^1	F_0	F_0	F
Day 3	32-37	69-71	F, E, L_1	F, E, L^1	F_0	F, E, L^1
Day 4	30-36	70-72	F, E, L_1, L_2	F, E, L_1	F, E, L^1	F, E, L_1
Day 5	31-38	69-72	L_1, L_2, L_3	E, L_1, L_2	L_1,L^2	F, E, L_1, L_2
Day 6	32-37	68-71	L_2, L_3	L_1, L_2, L_3	L_1,L^2	L_1, L_2, L_3
Day 7	33-39	67-70	L_3	L_2, L_3	L_1,L^2,L_3	L_2, L_3

Note: *F – Flies; E – Eggs; F_o - No flies; L_1, L_2, L_3 - Larval stages; T - Temperature; RH - Relative Humidity;M1- Fresh meat; M2 - Cooked meat; M3 - Salt and turmeric powder treated meat; M4 - Frozen meat.*

Identification of Dipteran Fly Larvae

The third stage dipteran fly larval stages (n=10) were randomly collected from the different types of mutton samples on the last day of the experiment i.e., the 7th day of the experiment. Thus, a total of 30 larvae from the three replicas of the experiments were randomly collected, processed, and identified. Based on the morphological features of the posterior spiracles, the larvae were identified. The larvae obtained from fresh mutton (M1) samples were of *Calliphora* spp. (60%) and *Lucilia* spp. (40%). The majority of the dipteran larvae in cooked mutton (M2) were of *Chrysomya* spp. (73.3%) and the remaining (26.7%) were of *Musca* spp. In the third group of salt and turmeric powder treated mutton sample (M3), the obtained larvae were identified as *Sarcophaga* spp. (n=16) and the rest of the larvae were of *Chrysomya* spp. (n=14). Contraryto the larvae identified in the third group (M3), the frozen mutton group (M4), noticed 50 percent of *Calliphora* spp. and the remaining were of *Lucilia* spp. (30%) and *Musca* spp. (20%) (Table 2). In the current study, posterior spiracles of third-stage dipteran larvae recovered from meat samples were identified by morphological features. Fresh mutton infested with four types of larvae viz., *Musca* spp., *Calliphora* spp., *Chrysomya* spp., and *Sarcophaga* spp. and with cooked meat, *Lucilia* spp., were observed. The current observations are comparable to the findings of Serbino and Godoy (2007) who conducted the experiments using chicken viscera, gizzard, and trapped the flies and noted that *Sarcophaga* spp. were abundant and *Musca* spp. counted low in number. In the current study, *Calliphora* spp., and *Chrysomya* spp. are the dominant larvae in mutton samples. The present observations corroborate with the findings of Nelder (2009) and Ekanem and Dike (2010). Contrary to present findings,Costa and Mendes (2014) noticed the larvae of *Musca domestica* alone. The size of the meat sample also has an effect on the abundance of the fly population. In the present study, a small sizeof ground meat sample (100 grams) was used to attract the flies. Silva *et al* (2014) proved in their research work that as the size of the carcass is large biomass, develops a higher abundance of flies. In the present study, meat with salt and turmeric powder treated samples attracted the flies, similar to fresh, cooked, or frozen meat samples, except with delay to attract the flies. Thus, the common kitchen ingredients (salt and turmeric powder) do not have any effect to prevent fly breeding. A study by Schmidt and Harris (1989) compared the production of larvae of *Chrysomya* spp. on ground beef and gelled medium containing 14 percent of beef and bone meal, 3 percent dried meat, and 3 percent non-fat fried milk, and observed more pupae in fresh meat. These findings concluded that fresh meat is having more preference over the other forms of meat.

Table 2: Type of fly larvae observed on different types of mutton

Type of chicken meat	Larvae observed (n=30)
Mutton –fresh (M1)	*Calliphora* spp. (n=18) (60.0%) *Lucilia* spp. (n=12) (40.0%)
Mutton –cooked (M2)	*Chrysomya* spp. (n=22) (73.3%) *Musca* spp. (n=8) (26.7%)
Mutton -salt + turmeric powder treated (M3)	*Sarcophaga* spp. (n=16) (53.3%) *Chrysomya* spp. (n=14) (46.7%)
Mutton -frozen (M4)	*Calliphora* spp. (n=15) (50.0%) *Lucilia* spp. (n=9) 9 (30.0%) *Musca* spp. (n=6) 6 (20.0%)

Observations on Quality Characteristics of Meat

The influencing of different physico-chemical attributes and proximate composition of different substrates of mutton significantly ($P<0.05$) influenced the breeding preferences of necrophagous flies (Table 3). A significant ($P<0.05$) higher water holding capacity (WHC) was found in the fresh mutton samples compared to the remaining formulations. The range of WHC (%) of different types of mutton substrates is between 29.48 to 51.55. The average pH of fresh meat samples was acidic in nature with slight variations between the different substrates of mutton. Cooked meat samples had significantly ($P<0.05$) lower water activity than the remaining substrates of mutton. Not much variation was noticed for the water activity between the meat samples and the value was close to one. The 2-TBARS values and percent FFA values were noticed high in cooked mutton than remaining formulations. The difference in 2-TBARS values and FFA values was considerably great between different types of mutton samples. Fresh mutton had significantly ($P<0.05$) higher moisture content and cooked mutton was found significantly ($P<0.05$) higher total fat and total ash content than the remaining formulations.

Table 3: Quality characteristics of various substrates of mutton

Quality Characteristics	Fresh	Cooked	Salt and turmeric powder treated	Frozen
Water Holding Capacity	51.55±0.44^{a}	29.48±0.20^{d}	47.30±0.29^{b}	40.66±0.35^{c}
pH	6.04±0.09^{c}	6.75±0.03^{a}	5.94±0.10^{c}	6.32±0.31^{b}
Water activity (a_{w})	0.98±0.09^{a}	0.89±0.14^{c}	0.93±0.23^{ab}	0.95±0.04^{a}
2-TBARS value	0.39±0.03^{c}	0.85±0.19^{a}	0.40±0.29^{c}	0.49±0.39^{b}
FFA value	0.175±0.03^{d}	0.498±0.14^{a}	0.327±0.16^{b}	0.276±0.09^{c}
Proximate Composition (%)				
Total Moisture	72.30±0.41^{b}	67.17±0.28^{c}	73.12±1.07^{a}	67.12±1.28^{c}
Total Protein	19.13±0.85^{c}	21.06±0.63^{b}	21.68±0.85^{a}	21.06±0.39^{b}
Total Fat	5.88±0.50^{c}	8.28±0.21^{a}	5.80±0.36^{c}	6.37±0.25^{b}
Total Ash	2.74±0.12^{b}	3.79±0.69^{a}	2.76±0.11^{b}	2.70±0.17^{b}

Note: *Average mean ± SE values (n=6); 2-TBARS-2-Thiobarbuteric Acid Reactive Substance; FFA- Free Fatty Acids; Values with different superscripts differ significantly (P<0.05)*

Fresh mutton had significantly (P<0.05) higher water-holding capacity than the remaining substrates of mutton. Further, there is considerable variability in water-holding capacity between differentsubstrates of mutton. This variability is due to differences in processing variables with similar characteristics that showed large differences in water-holding capacity (Lawrie 1991). Generally, meat from freshly killed animals has an average pH of 6.8, which falls rapidly to 5.4- 5.6 in a duration of 48 hours post-mortem. The level of pH attained again depends upon which type of processing method and type of muscle and temperature during processing. Ziauddin (1994) observed thatthe biceps femoris muscle with initial pH of 6.97 had an ultimate pH value of 6.0. The processing variables like cooking and freezing significantly (P<0.05) influenced the water activity. Usually fresh and pre-rigor meat obtained from different species is not differ in any water activity levels (Kristensen and Purslow (2001). Generally cooked samples of mutton had higher 2-TBARS values and FFA values than the remaining types of mutton. The higher 2-TBARS and FFA values in cooked mutton might be due to more lipid oxidation which was initialed by the cooking of mutton thus fat was more unstable and easily oxidized. The magnitude oflipid oxidation favours the compositional changes of the meat and generates different off odours. The values of free fatty acid are a measureof fat status and quality. The variations in the fly preference for different types of meat samples might be due to the variations in pH, water activity, and 2-TBARS values. However, the values of the chemical composition of different substrates of mutton are not the same. The variations in the proximate composition of different types of mutton might be due to differences in the type and quantity of extracted protein and protein-cation interaction. Divalent cations are thought to reduce meat stability by increasing protein aggregation through the formation of salt bridges (Asghar 1985).

Conclusions

The present study envisaged that a high degree of control canbe achieved by adequate attention to hygiene which may include the proper disposal of carcasses and slaughterhouse offal. Irrespective of the type of mutton substrates and their form and physico-chemical properties attract the carrion flies. These kinds of non-chemical fly suppression measures require field assessments as the laboratory results may not always be reliable indicators of field performance. Further studies are required to explore the fly attractants for the target species that need to be assessed for the on-field application so that, the ecosystem is protected without disturbing the beneficial insects.

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Contribution by authors

All the authors contributed equally to writing the manuscript. The final manuscript was read by all others and consented to publication.

Conflict of Interests

There is no conflict of interest.

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