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RELATIONSHIP BETWEEN CARBON DIOXIDE (CO^2) AND POPULATION OF AIRBORNE MICROORGANISMS IN CATTLE FARM AT LADANG PASIR AKAR, TERENGGANU, MALAYSIA

NUR SYUHADA AHMAD NORDIN[1], AZMAN AZID*[1], MOHD SAIFUL SAMSUDIN[2], KU MOHD KALKAUSAR KU YUSOF[3] AND FATHURRAHMAN LANANAN[1,4]

[1]Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut Campus, 22200 Besut, Terengganu, Malaysia. [2]Faculty Business and Entrepreneuship, Universiti Malaysia Kelantan, Kampus Kota, Karung Berkunci 36, Pangkalan Chepa, 16100 Kota Bharu, Kelantan, Malaysia. [3]Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21300, Kuala Nerus, Terengganu, Malaysia. [4]East Coast Environmental Research Institute, Universiti Sultan Zainal Abidin, Gong Badak Campus, 21300 Kuala Nerus, Terengganu, Malaysia.

**Corresponding author: azmanazid@unisza.edu.my*
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Abstract: Carbon dioxide (CO_2) is essential for environmental balance because animals release CO_2 and then plants uptake it for photosynthesis. The aim of this study is to identify the concentration of CO_2 and microorganism population in cattle farming and to determine the relationship between these two variables. Aeroqual 500 series sensor was used to determine CO_2 concentration, while DRF e-MAS was used to detect microorganisms. *Escherichia coli* (*E. coli*) is one of the possible bacteria found in the microorganism population, and catalase tests were performed to determine this. Results showed *E. Coli* to be present in the microorganism population. Based on the correlation analysis, there was no relationship between CO_2 and the microorganism population in the cattle farm.

Keywords: Carbon dioxide, Cattle farm, *E. coli*, Indoor environment, Microbial.

Introduction

CO_2 is considered the most powerful greenhouse gas (GHG) as it has the most direct-warming impact on global temperature. The primary sources of CO_2 emissions in cattle farms are soil, feed crops and animal respiration, with a smaller contribution from microbial manure respiration (Chianese *et al.*, 2009). Soils and cultivation land for feed crops can release millions of tons of CO_2 per year. However, there was a consistent diurnal pattern in CO_2 emissions from the open-lot area with lower emissions throughout late evening and early morning and then rises during the day, with maximum late-day levels. Wind speed and temperature will link the diurnal pattern. Winds tend to be light in the late evening and early morning and then increase steadily throughout the day to reach a peak level. Temperature also rises from early morning to late afternoon and then decreases again (Leytem *et al*., 2011). Cattle behavior tends to increase from morning to late afternoon as they wake up, feed, drink, ruminate and urinate on the field. More energy needed; more CO_2 produced.

Cattle manure is known to harbour a wide variety of microorganisms that can be pathogenic or non-pathogenic to both animals and humans, through microbial respiration, can contribute to CO_2 emission. Excessive CO_2 emissions can increase the current earth surface temperature that can affect the health of cattle, reduce reproductive efficiency in both males and females, and decrease feed conversion efficiency (Lees *et al.*, 2019). Heat stress is one of the common ailments in cattle due to high temperature. Heat stress can cause growth rate reduction, prolonged puberty time, and low milk production per lactation. Embryonic mortality and high mortality in cattle itself can be worse.

Microorganisms can be found from containment buildings in bio-aerosols. Cattle produces a large quantity of manure consisting of feces and urine along with undigested feed and other secretions, such as vaginal, mammary gland and nose secretions. The levels and types of microorganisms in cattle waste vary depending on their dietary sources, cattle's health status and age, manure's physical and chemical

characteristics, and manure storage facilities. The survival rate of each microorganism ranges from a few days to several months depending on the species being monitored and their ability to adapt to the animal manure's hostile atmosphere into which they excreted. Microorganism survival period also influenced by favourable temperature, pH, moisture content, nutrient availability or organic content, biological interactions, time and organism density in manure (Manyi-Loh *et al*., 2016).

The aim of this study is to identify CO_2 concentration and microorganism population with *E. coli* in cattle farms and to determine the relationship between these two variables.

Materials and Methods

Study Area

Assessment was carried out in the cattle farm at Ladang UniSZA Pasir Akar, Terengganu, Malaysia (Latitude: 5.643865; Longitude: 102.471009). Figure 1 shows the study location. Due to the possibility of high concentration of CO_2 emissions and microbial aerosols, this area was chosen as a sampling site. This farm used an open feedlot system with a total area of 1,494 m^2 and a perimeter of 197 m. Open feedlots are good to ensure better ventilation for cattle. According to the School of Veterinary Medicine (2015), whatever system is chosen, it must provide fast moving air in the cattle's resting area, especially during the hot season. Ventilation provides fresh air to the building space that displaces contaminated, warm and humid air. Without proper ventilation, the cattle will be at risk to heat stress and poor respiratory health.

Assessment of Carbon Dioxide

The CO_2 concentration was measured using the Aeroqual 500 series sensors. This device allows users to read samples quickly and efficiently. The CO_2 sample was collected using Aeroqual 500 series sensors at four points inside the cattle barn. Measurement position at each point was 1.5 m from the ground (Yasmeen *et al.*, 2019). This is because 1.5 m is the average human and cattle breathing height. Most of the cattle raised on this farm were imported breeds – Brahman and Angus – about 1.5 m high. The sampling time for each point was approximately 30 seconds.

Assessment of Microorganisms' Population

A microbial air monitoring systems, known as DRF e-MAS (Figure 2), was used to assess the microorganisms' airborne population. DRF e-MAS was used as an alternative to HACH MAS-100, which used the standard approach for evaluating biological pollutant according to USEPA CPSC # 425 during production of this equipment. DRF e-MAS was designed as a 3-in-1 air sampler with a plate chamber for biological sampling with a petri dish of 60 mm and 150 mm diameter and heavy metal sampling with 50 mm filter paper. The open-source Arduino microcontroller is used to promote prototyping and integration of wind speed algorithm and

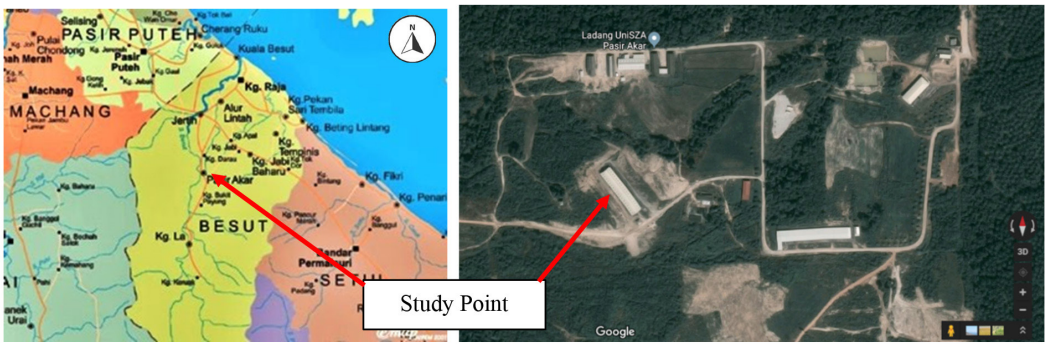

Figure 1: Map of the study area in Ladang UniSZA, Pasir Akar, Terengganu

Figure 2: Microbial Air Sampler (DRF e-MAS)

accuracy control. Two-type power source, AC and DC, from an internal lithium polymer battery for field sampling was used.

The plate chamber aspired air with a maximum volume of 1.74 m^3/min that circulated through the plate chamber. The measurement was taken at 1.5 m from the ground for every four points inside the barn. Nutrient agar prepared a day before sampling was put inside the DRF e-MAS plate chamber. Time taken at each point was about 30 seconds. After 30 seconds, the nutrient agar was sealed and incubated at 37 °C for 24 hours. After 24 hours, the nutrient agar population was counted and the result was recorded.

Escherichia coli Identification

According to Rahn *et al.* (1997), Manyi-Loh *et al.* (2016), Stein and Katz (2017) and Islam *et al.* (2019), airborne microorganisms such as *E. coli* are commonly found in indoor cattle barns. Generally, the individual cattle are temporarily colonized and *E. coli* **is shed in their faeces.** *E. coli* is the bacteria present in air droplets from commercial beef processing plants and can be spread by air (Stein & Katz, 2017). Therefore, this study focuses more on *E. coli*.

E. coli was collected using DRF e-MAS. DRF e-MAS measuring height during sampling was 1.5 m, the same as when CO_2 collected. MacConkey agar prepared a day before sampling was used and placed inside the DRF e-MAS plate chamber. Time taken during sampling was approximately 30 seconds, immediately sealed and incubated at 37 °C for 24 hours. After 24 hours, *E. coli* rapid lactose-fermenting colonies appeared on MacConkey agar. MacConkey agar contains cholate and taurocholate bile salts as Gram-positive flora inhibitor and lactose-neutral red as acid production indicator. It is also used not only to inhibit Gram-positive organisms and yeast, but also to differentiate Gram-negative organisms by lactose fermentation (Wanger *et al.*, 2017; Singh *et al.*, 2017). Donut-shaped and dark pink spots appeared on the MacConkey agar, surrounded by dark pink precipitate bile salt (Sah *et al.*, 2017). This observation indicated *E. coli*'s presence on the MacConkey agar. *E. coli* is an anaerobic, Gram-negative bacilli that ferments lactose to produce hydrogen sulphide (Yaratha *et al.*, 2017). Using the inoculation loop, single colony of *E. coli* on MacConkey agar was picked up and streaked to another nutrient agar to ensure that *E. coli*'s growth continued.

The nutrient agar with *E. coli* was incubated for another 24 hours at 37 °C. After 24 hours, a single *E. coli* colony was picked up for catalase testing. Then, the colony was taken and smeared on a glass slide. Three drops of hydrogen peroxide were applied on the colony. The presence of gaseous bubbles indicates a positive result (Saadi & Hussein, 2017).

Statistical Analysis

Both CO_2 sample and microorganism population assessment were collected four times. All data were analyzed statistically using Microsoft Excel. Microsoft Excel's descriptive analysis was applied to answer the first objective. Descriptive analysis is used to define the basic data features in a study by offering concise summaries of the sample and measurements. This study identifies outliers and compares distributions between CO_2 and airborne bacteria. The findings were presented in a box and whisker plot, which signifies the descriptive statistics of the data set (Samsudin *et al*., 2019a). The "stem and leaf diagram" in the box plot represents the

data semi-graphically (Samsudin *et al.*, 2019a; 2019b).

Both CO_2 and airborne bacteria population finding data were also incorporated into the correlation analysis. Correlation is a statistical method for evaluating a possible linear relationship between two continuous variables (Mukaka, 2012). A high correlation means that there is a strong relationship between two or more variables, while a low correlation means the variables are hardly related. While a t-test was used to test whether there is a difference between two independent sample means, when there is only one sample (Kim, 2015). To establish the relationship between CO_2 concentration and the population of microorganisms in Ladang Pasir Akar's cattle farm, correlation analysis and t-Ttst were applied to answer objective number two.

Results and Discussion

Descriptive Analysis of the Concentration of Carbon Dioxide and Microorganisms' Population

To identify the concentration of both CO_2 and microorganism population in the cattle farm, the descriptive analysis was used to summarize the raw data collected as mean, minimum value and maximum value. Table 1 presents the counted concentration of CO_2 and bacteria. Whereas, Figure 3 shows the CO_2 and bacteria concentration in boxplot.

Table 1: Descriptive statistic of the CO_2 concentration

	CO_2 (mg/m^3)	**Bacteria count (cfu × 10^3/m^3)**
Mean	108.0	62.1
Minimum	97.2	17.3
Maximum	122.4	141.5

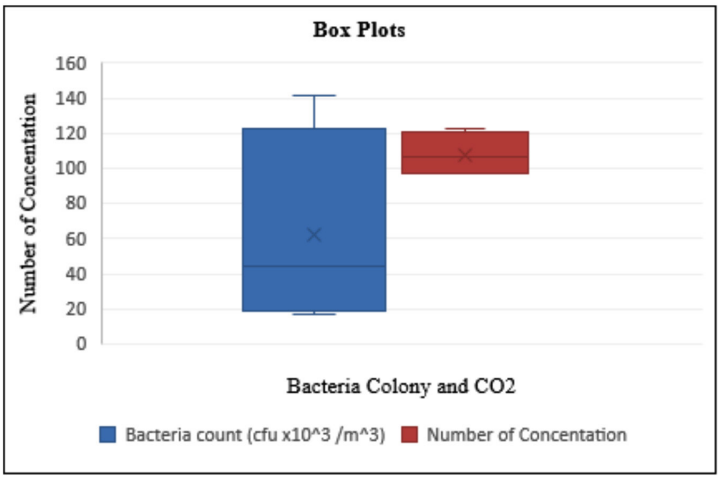

Figure 3: Box plots of the concentrations of CO_2 and bacteria population

The results indicate the presence of CO_2 concentration in the cattle farm. CO_2 average was 108.0 mg/m^3. The CO_2 minimum and maximum were 97.2 mg/m^3 and 122.4 mg/m^3, respectively. CO_2 concentration was higher at the centre of the barn with more than 50 cattle, including calves. CO_2 concentration at 1.5 m is higher, because most adult cattle with this height are located inside the barn compared to another 13 calves. Nonetheless, CO_2 emission value is lower than permitted value in a building. According to Wisconsin's Department of Health Services (2019), the volume of CO_2 in a building is typically related to how much fresh air is brought into the building. Furthermore, the density inside the cattle barn is low, as each lot is divided to not more than 20 cattle and organized according to their physical state such as

lactating, pregnant cow, non-pregnant and ready for mating. Each cattle in each lot had enough space to continue their daily lives. According to the U.S. Department of Agriculture (2019), barn space is 1.9 to 2.8 m^2 for cattle weighing 454 to 590 kg, and cattle have access to a lot. Brahman's average body weight is 454 to 635 kg while Angus is 454 to 544 kg. As a result, the overall density inside the cattle barn is low. Thus, CO_2 concentration does not exceed the permitted value.

Animal respiration and microbial respiration in manure are primary sources of CO_2 emission (Chianese *et al.*, 2009). Fresh manure accumulation was observed during sampling. Approximately 90% of Ladang Pasir Akar's barn floor was covered with manure and can contribute to CO_2 emissions. The sample was also collected from 10 am to 12 pm. According to Leytem *et al.* (2011), CO_2 emissions will increase from morning to afternoon due to animal behavior, including feeding, drinking, ruminating and urinating. The maximum CO_2 emission value was also collected during tractor entry into the cattle barn to bring cattle grass. It is because this farm follows an intensive management system, where cattle are in confinement and are dependent on humans to meet every day basic needs such as food, shelter and water. Koneswaran and Nierenberg (2008) reported CO_2 emissions in cattle farming due to the high amount of fossil fuel burned, particularly for machinery and transport. Leytem *et al.* (2011) also stated that average daily emissions from open lots should be 637 g CO_2. Mohd-Firdaus & Juliana (2014) said the CO_2 exposure limit is 1800 mg/m^3 as recommended by the National Institute for Occupational Safety and Health (NIOSH). The maximum value of CO_2 in cattle did not exceeded the exposure limit.

Based on the data collected, the mean value of the population of microorganisms was 62.1 cfu × $10^3/m^3$. The minimum value was 17.3 cfu × $10^3/m^3$, while the maximum value was 141.5 cfu × $10^3/m^3$. During sampling, temperature and humidity are observed inside the cattle barn using temperature and humidity detector (Fisherbrand™ Traceable™). Based on the results, the higher humidity level, the higher the value of the farm population of microorganisms. Moreover, it was the rainy season during the sampling day that can contribute to a high percentage of humidity in the cattle farm. According to Manyi-Loh *et al.* (2016), the high moisture content in manure can serve as a microorganism reservoir. High temperature is not a suitable habitat for growing microorganisms. Nevertheless, some bacteria can die at high temperatures in the environment (Wang *et al.*, 2004). The sampling results show the higher temperature in the cattle barn, the lower value of population of microorganisms.

Correlation Analysis

A correlation analysis was conducted to identify the relationship between CO_2 and microorganisms in the cattle farm. Table 2 and Table 3 show correlation findings in detail. R-value was 0.2081. According to Table 3, the R-value was below 0.3, suggesting a weak relationship. There is no relationship between CO_2 and microorganism population in the cattle farm.

Table 2: Correlation between microorganisms' population and CO_2

	Bacteria count (cfu ×$10^3/m^3$)	**CO_2 (mg/m^3)**
Bacteria count (cfu ×$10^3/m^3$)	1	
CO_2 (mg/m^3)	0.2081	1

Table 3: Strength of linear relationship

Correlation coefficient value, r	Strength of linear relationship
At least 0.8	Very strong
0.6 up to 0.8	Moderately strong
0.3 to 0.5	Fair
Less than 0.3	Poor

A t-test analysis was performed to identify the significant difference in the relationship between CO_2 and microorganism population in the cattle farm. Table 4 shows the t-test results. Based on the result, the p-value was 0.10. Since the p-value is larger than 0.05, so it fails to reject the null hypothesis, and cannot conclude that a significant difference exists. Thus, there is no significant difference between CO_2 and microorganism population on a cattle farm.

Table 4: Significant difference of relationship between CO_2 and population of microorganisms in cattle farm

	CO_2 (mg/m^3)	Bacteria Count (cfu ×10^3/m^3)
Mean	108.0	62.1
Variance	164.16	3272.19
Observations	4	4
Pearson Correlation	0.2081	
Hypothesized Mean Difference	0	
df	3	
t Stat	1.64	
P-value	0.10	
t Critical	2.35	

Catalase Test for E. coli

Based on the catalase test, Table 5 shows all positives, which mean, there are *E. coli* inside the cattle farm. According to Manyi-Loh *et al.* (2016), *E. coli* is one of the presumptive bacteria that is commonly found in cattle farms. *E. coli* also has been reported as the most notorious pathogen which produces a potent toxin that can cause serious infection in humans. However, cattle harbouring *E. coli* strains do not develop clinical disease, but serve as the main reservoir for *E. coli*. Moreover, the main reservoir for *E. coli* is the intestinal tracts of healthy cattle. Individual cattle are transiently colonized and shed *E. coli* in their feces. During sampling day, there are large accumulations of feces on the barn floor. This situation can help *E. coli* build a reservoir and grow. On the other hand, according to Alam and Zurek (2004), one of the potential modes of transmissions for *E. coli* in the environment is by houseflies that are associated with animal faeces and manure. Houseflies build up a very large population in cattle farms and commonly ingest *E. coli* and transmit it from a barn to another barn. During our sampling, many houseflies flew around the barn and alighted on the cattle.

Table 5: Catalase test for *E. coli*

P1	P2	P3	P4
+	+	+	+

Conclusion

Based on the descriptive statistical analysis performed to analyse and summarize the raw data, it shows that the maximum CO_2 value in the cattle farm did not exceeded the exposure limit. Determination of the relationship between CO_2 and microorganism population in Ladang Pasir Akar's cattle farm was proven using correlation analysis. Since this barn utilised an open feedlot area, there was no relationship between these two variables. Thus, CO_2 emissions on farm did not affect the total microorganism population and vice versa. Catalase test was performed to identify *E. coli*'s presence in the barn.

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