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A dissertation submitted in fulfilment of the requirements for the degree of

Master of Science in Agriculture (Animal Science)

With the provisional title:

Effect of feed rations on livestock performance and impact of effective microorganisms on litter odour and other gases emissions.

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DECLARATION

I declare that this dissertation, is the product of my work and effort, the results are my independent investigations and research. I further, declare that all sources of information used or quoted were acknowledged.

I acknowledge that I have read and understood the University's' policies and rules applicable to postgraduate research, and I certify that I have, to the best of my knowledge and belief that I have complied with all the requirements.

Signed by:

Victor Welcome Sithole

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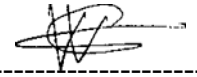

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ABSTRACT

The increase in demand for animal protein has increased the demand for pork and beef by humans to supply this dietary protein. The short gestation period as well as the high number of piglets renders pig production relatively viable, hence increasing the number of farms. Feedlot farming has increased this production but not without high waste and odour emission which may be hazardous to animals, workers and the surrounding community members. Therefore, the aim of this study was to evaluate the effect of feed rations on livestock (cattle and swine) performance and the impact of effective microorganisms (EM) on litter type odour and other gaseous emissions. It was hypothesized that diet type and EM would not livestock litter odour and gaseous emissions. Experimental chapter one and two investigate the effect of feed type and effective microorganisms on performance and litter odour emission from feedlot cattle and swine, respectively. The EM was applied on litter at different treatment levels (10% EM, 20% EM, and 30% EM) to mitigate odour emissions. The results showed that different diets (starter, grower and finisher) fed to both cattle and swine improved ($P<0.05$) performance as the feed intake, weight gain and feed conversion ratio increased when animals changed from one diet to the other. However, it was also noted that the diet affected ($P<0.05$) odour emission from both beef cattle and swine manure. This was because the gaseous emission increased ($P<0.05$) as the beef animal's diet changed from starter to grower while emissions from pig was also different ($P<0.05$) between starter and finisher. The gaseous compounds identified were classified into; Alcohols (4), aldehydes (6), volatile fatty acids (13), ketones (3), terpenoids (2), amides (3), phenolics (8), sulphur compounds (2) and nitrogen containing compounds (3) from beef litter. From pig litter, the gaseous compounds identified were classified into alcohols (5), aldehydes (5), volatile fatty acids (13), ketones (2), terpenoids (2), amides (1), phenolics (7), sulphur compounds (3) and nitrogen containing compounds (3). Among

these gases, P-Cresol (4-methyl phenol) and phenol was assumed to have the highest potential hazardous effect in terms of air pollutants as listed by the US Environmental Protection Agency. Indole and skatole were also identified and have often been associated with acute bovine pulmonary edema and emphysema (ABPE) in cattle and boar taint in pigs. Effective microorganisms (EM) treatment showed no differences ($P>0.05$) among all litter treatment levels (10% EM, 20% EM, and 30% EM) but for the control. There were significant differences in the odour emitted between the different weeks ($P<0.05$) when compared to the control. P-Cresol, phenol, indole and skatole were reduced by EM to undetectable amounts in week 3 compared to the controls. However, dimethyl sulphide and dimethyl trisulphide were not mitigated by the use of EM hence further methods on how to mitigate these sulphur compounds need to be explored. A 10% dose of EM for cattle litter treatment was preferred, since the effect of higher doses was minimal. However, for swine, a 30% EM dose treatment was recommended since most of the odorous compounds (Indole, skatole, phenol, and dimethyl sulphide and dimethyl trisulphide) were reduced to undetectable amounts. It was also concluded that EM does not depend on concentrations to reduce odour from livestock manure, but time for the microbes to adapt, grow and multiply to mitigate odour.

Keywords: Effective microorganisms, odour, volatile organic compounds, diet, P-Cresol, phenol, indole, skatole, dimethyl sulphide, dimethyl trisulphide, boar taint.

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TABLE OF CONTENTS

LIST OF TABLES

WORK FROM STUDY PRESENTED IN A CONFERENCE

1. Sithole, VW. Fon, FN. & T.N. Suinyuy. 2019. Effect of feed rations on livestock performance and impact of effective microorganism on litter odour and other gases emission: A review. Presented at the 51st South African Society for Animal Science (SASAS) Congress which was held at the University of the Free State in Bloemfontein, Free State, South Africa from 10 to 12 June 2019.

PAPERS SUBMITTED FROM THE THESIS FOR PUBLICATION

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WORKING ON MANUSCRIPTS FOR PUBLICATIONS

1. Sithole, VW. Fon, FN. & T.N. Suinyuy. Effect of feed type and effective microorganisms on feedlot cattle performance and litter odour emission, respectively.

2. Sithole, VW. Fon, FN. & T.N. Effect of feed type and effective microorganisms on pig performance and litter gaseous emissions, respectively.

LIST OF ABBREVIATIONS

Abbreviation	Description	units
EM	Effective Microorganisms	ml
DM	Dry Matter	%
MC	Moisture Content	%
OM	Organic Matter	%
NDF	Nutrient Detergent Fibre	%
ADF	Acid Detergent Fibre	%
HEM	Hemicellulose	%
CP	Crude Protein	%
Ca	Calcium	%
Mg	Magnesium	%
K	Potassium	%
Na	Sodium	%
P	Phosphorus	%
Zn	Zinc	ppm
Cu	Copper	ppm
Mn	Manganese	ppm

Fe	Iron	ppm
SEM	Standard Error of Means	None
AOAC	Association of Official Analytical Chemists	None
SAS	Statistical Analysis System	None
CAS	Chemical Abstract Service	None
KRI	Kovats Retention Index	None
VOCs	Volatile Organic Compounds	None
AA	Amino Acids	None
VFAs	Volatile Fatty Acids	None
CO_2	Carbon Dioxide	None
N_2O	Nitrous Oxide	None
H_2S	Hydrogen Sulphide	None
NH_3	Ammonia	None
CH_4	Methane	None
LAB	Lactic Acid Bacteria	None
PNSB	Purple non-Sulphur Bacteria	None
KZN	Kwa-Zulu Natal	None

EPA	United States Environmental Protection Agency	None

CHAPTER ONE: General Introduction

1.1 Background

Often, when people are driving by a livestock operation or farm, they suddenly start winding up their car windows or close their nostrils because of the odour coming from litter. Livestock operations have been facing complaints from local residents, workers and hospitals for environmental pollution. There are even claims that besides the stuffiness of the odour, it might contain poisonous gases which could cause serious health problems such as headaches, sinus, coughing, eye irritation, dizziness and even death with prolonged exposure (Rappert and ller, 2005, Kailene and Warrior, 2019). Hotels and restaurant owners also claim that these bad smells drive away their customers (Peters, 2014b). These gases do not only affect human beings but may also affect animals within these operations by causing certain ailments, such as respiratory diseases that may negatively affect feed intake, weight gain, and the overall performance of the animal (Bibbiani and Russo, 2012, Akdeniz et al., 2012). Temporary solutions have been consistent such as cleaning, moving the farm far from the community or residential areas, which are both relatively expensive. Other methods include composting and making use of heat spray to reduce the gases from livestock. These methods also either consume a lot of energy, are expensive or time consuming (Bastami, 2016). There is a need to find solutions which are less expensive and user friendly for both subsistence and commercial farmers. Hence, the potential of effective microorganisms (EM) in the reduction of toxic gases from livestock operations should be explored.

1.2 Justification

The use of effective microorganisms in the treatment of animal waste in this study will generate information on the treatment of pig and cattle waste for odour control in livestock production systems, which are still a major problem today. This information will help in improving sustainable production and protection of the environment. Optimization of the use of effective microorganisms on pigs and beef cattle will help to improve the economic, nutritional and social status of pig and cattle production farmers in South Africa.

1.3 Objectives

The broad objective of the study was to evaluate the effect of feed rations on livestock performance and the impact of effective microorganism on litter odour and other gas emission. The specific objectives were:

1. To investigate the effect of feed type and effective microorganisms on feedlot cattle performance and litter odour emissions, respectively.

2. To determine the effect of feed type and effective microorganisms on pig performance and litter odour emissions, respectively.

1.4 Hypotheses

The hypotheses tested were that:

1. Feed type and effective microorganisms will not improve feedlot cattle performance and decrease litter odour emissions, respectively.

2. Feed type and effective microorganisms will not improve pig performance and decrease litter odour emissions, respectively.

CHAPTER TWO: Literature review

2.1 Introduction

One of the main issues challenging farmers and municipalities worldwide is the treatment, disposal and recycling of solid waste from agricultural practices. This is in addition to the solid wastes that are gases emitted, which may be odorous or odourless with potential health hazard or environmental pollution. The gaseous emissions that may result from three basic sources are; animal buildings (30%), fertilizer storage facilities (20%), and animal litter storage or application on the fields as manure (50%) (Rappert and Müller, 2005). As the amount of large livestock operations increase, complaints about the odours from livestock have also increased, especially in swine operations where animal concentration increases in small geographic areas (Rappert and Müller, 2005). The use of effective microorganisms has been tested by different researchers as an attempt to decrease the spread of odour in the environment (Higa, 1991, Chantsavang and Watcharangkul, 1998, Freitag and Meihoefer, 2000, Li and Ni, 2001, Mafiri, 2014). Therefore this review discusses the contribution of livestock to odour production. Particularly, the challenges faced by feedlot cattle and pig industries and their attempts to control waste odour emissions and specific odour sources. The review also includes the different methods that have been used to control gaseous and odour emissions, for example, effective microorganisms.

2.2 Contribution of cattle to odour production

As has been mentioned earlier, the control of odour in livestock production systems is a major problem. In South Africa, the Nguni people (Swati, Zulu and Xhosa people) have relied on cattle farming for centuries. However, the system has changed to more intensive farming operations due to the high demand for animal products, such as meat and milk. The main objective of these

intensive types of production is to meet the high demand of livestock products (Archer van Garderen, 2011). As these feedlots systems intensify, the amount of excreta produced also increases, resulting in a higher level of pungent odours. This has resulted in many countries reporting and complaining about bad odours being emitted from different livestock production systems (Mielcarek and Rzeznik, 2015). In April 2000, the United States Environmental Protection Agency (EPA) released a notice of violation under the clean air act against seven premium standard factory farms in Missouri, indicating that they released odours that contain harmful gases (Karvosky, 2006). These animal emissions from the factory were threatening the health of neighbouring businesses, which eventually led to the closing down of a day-care centre near the farm in question. In fact, the children and carers were instructed to vacate the premises after it was discovered that they were suffering from symptoms of hydrogen sulphide poisoning (Karvosky, 2006).

In 2014, Triple A, one of the largest feedlots and abattoirs in South Africa (KZN) had been taken to the Pietermaritzburg high court, fighting against their closure due to bad odours being emitted from the feedlot. People located near the farm sought an interdict against Triple A Beef (which has between 24 000-27 000 cattle on its property), to stop it from operating as a feedlot and abattoir as a result of the mass generation of odour from cattle manure (Peters, 2014a). The manure from places such as these result in the production of flies, insects, and odours that consist of undesirable gasses in an urban environment. Peters (2014a) stated that residents from informal settlements and hospitals located about 100m and 20km away had complained that agricultural practices at Triple A had polluted the air, which posed serious health risks to the people causing respiratory problems resulting in headaches and nausea.

2.3 Contribution of pigs to odour production

The increased demand for animal protein by humans has paved the way for growth in pork production, since beef is relatively more expensive. The increase of swine in large livestock operations has resulted in complaints about livestock odours, especially as intensive systems increase in small geographic areas (Rappert and Müller, 2005). Intensive production systems produce large quantities of excreta, which increase the amount gaseous odours emitted from piggeries (Mpendulo, 2012). The odours emitted from piggeries reduce air quality resulting in air pollution. These emissions come from a composition of urine, fresh decomposing faeces, spilt feed, manure and waste storage handling systems, such as lagoons (Schauberger et al., 2006). Therefore, complaints from people located near these farms is obvious. Complaints due to agriculture-related odours have been reported to increase in many countries to about 20% yearly, approximately 40% of the complaints are from poultry farms and 35% from pig farms (Mielcarek and Rzeznik, 2015). In 2015, over 500 residents living near one of the largest piggery farms in North Carolina, United States of America, filed a lawsuit against Murphy Brown, which is a pig production farm of the Virginia-based meat conglomerate Smithfield Foods. These complaints arose owing to the high emissions from lagoons that disturb their lives and reduce the value of their properties. One couple stated that "they were forced to close down their family business restaurant" since the odour drove away their customers (Kuo, 2015). Other residents stated that they felt like prisoners in their own homes because they were unable to open their windows. They were forced to stay indoors because the first thing that they inhaled when they woke up was the odour of hog excreta from across the street. The odour of rotten eggs filled the residents yards at least twice a week resulting in nausea, difficulties in breathing and vomiting (Wing and Wolf, 2000, Kuo, 2015). Health problems such as burning eyes, nasal irritation, coughing, dizziness,

dyspnoea, chronic bronchitis, and wheezing were observed from employees of pig CAFOs (Concentrated Animal Feeding Operations), particularly those working with liquid manure (Cole et al., 2000).

In April 2017, residents living in Hayfields South Africa (KZN), sent numerous emails and complaints to a piggery farm named Baynesfield Estate which had about 1600 sows, saying that the pungent smell was so strong that residents were forced to close their windows. Residents complained that they could barely breathe and this resulted in sore throats, coughing, sinus problems and burning eyes (Kailene Pilay and Warrior, 2017). Farmers have long been in battle over how animal waste should be managed. According to the North Carolina Pork Council, the industry accounts for approximately R8 billion a year in income and 46,000 full-time jobs in production and processing, making the state the second largest pork producer in the US (Ernst, 2006). Such industries cannot easily close down or relocate because odour pollution will continue to be a problem wherever they are located, since odour diffusion and distribution by wind will continue to be a problem (Mielcarek and Rzeznik, 2015). Hence, it is critical to find an alternative solution on how to manage the animal waste to reduce the odour emission rather than changing the location of farms.

2.4 Environmental effects of pig and beef cattle odour emission

Pig manure may contribute to the negative environmental effects of greenhouse gases by increasing its concentration of emissions, hence reducing the air quality. Environmental pollution is not limited to gases, but water quality has also been found to be compromised by large amounts of excretions and effluents from intensive pig production systems. The waste produced from piggeries is either in solid or gaseous form. Soil and water quality is affected by the solid form, while the gaseous form reduces the air quality (Mpendulo, 2012). A majority of the affected

population located near piggeries or feedlot farms lack the sufficient knowledge and access to the correct channels in order to complain about the odour issues. These populations are unclear as to who they should approach with these concerns, and what protocols should be followed to address their complaints (Zaini and Lukman, 2015). Hence, it is crucial to understand the hazards that pig slurries pose to the environment with a mind to assist policy-makers develop strategies that will enforce commercial farmers to create the means to reduce environmental loads from piggeries (Mpendulo, 2012). This will help minimize the negative effects of pig waste on water quality, air quality and emission of greenhouse gases (Mpendulo, 2012).

2.4.1 Water quality

Farmers practice nutrient re-cycling by using pig or cattle manure to help increase crop growth or feed it to fish kept in ponds. However, the overuse of livestock has resulted in the seepage of nutrients through the soil to reach the groundwater, hence also destroying the soil structure (Mpendulo, 2012). Moreover, lagoon breaks have resulted in the discharge of millions of gallons of animal excreta directly into the water surface at once, resulting in eutrophication, the death of fish and a high load of environmental pathogen. The extreme leakage losses from lagoons has been reported to cause groundwater contamination with nitrates, while the microbes have resulted in the pollution of drinking water (Cole et al., 2000). Another type of contamination are nitrates, which leak out of fertilizer (manure) storage facilities into the source of community drinking water with the resulting seepage from animal feedlots, lagoon systems and land areas where liquid animal waste or manure has been applied. Nitrates above 10 milligrams per litre in drinking water can lead to human health risks, such as methemoglobinemia or “blue baby” disorder, which can result in developmental deficiencies or even death. Children younger than five years old, the elderly and people with suppressed immune systems are mainly affected by this disorder (Florida et al., 2008).

This problem needs to be monitored to reduce the negative impact that affect the residents located nearby commercial farms.

2.4.2 Air quality

Air quality is the degree of pollution of clean air, which is air that is free from contaminants. Measuring the concentration of pollutants in the air can be used to determine air quality. Hence, for better air quality, the number of airborne pollutants should be in low concentration (Wang and Zhang, 2010). Air pollutants are generally defined as the compounds or substances that when mixed with air reduce the air quality. Normally, air consists of 78.09% nitrogen, 20.95% oxygen, 0.93% argon, 0.04% carbon dioxide, and small amounts of other gases (Horn, 2018). The excessive concentration of other gases such as volatile organic compounds (VOCs), ammonia, nitrous oxide, carbon dioxide, hydrogen sulphide, and methane may lead to reduced air quality, causing odours and resulting in air pollution. Feeding operations such as feedlots can affect air quality through the emission of gases from animal wastes such as ammonia and hydrogen sulphide (Prapaspongsa, 2010), which often result in an unpleasant odour. The gases and odours produced from cattle and pig manure ensue from decaying matter (1) after it is produced, (2) during storage and treatment and finally (3) during land application of manure as fertilizer (Casey et al., 2006).

The pork industries are responsible for excretions of over 1.6 million tons of waste a year (Hribar and Schultz, 2010). Even though pig waste (pig manure/pig slurry) is a valuable resource to farmers in terms of providing nutrients and energy for crops, improper management of the manure can harm the environment via different kinds of emissions with potential air pollution, which has been highlighted above. Volatilization of ammonia occurs when the pig manure is applied on land (Hribar and Schultz, 2010), resulting in emissions and reduced air quality in the environment. Lagoons are mostly used for storage and management of livestock waste. As the waste increases

in size, treatment and management becomes necessary. The majority of swine lagoons rely on anaerobic bacteria (bacteria that does not use oxygen) to decompose the organic matter because more organic matter per unit lagoon volume can be handled by anaerobic bacteria than aerobic processes (Cole et al., 2000). The anaerobic reaction that occurs when the manure has been stored in the lagoons for a long period of time is the main cause of the odours produced. The odour is then carried away from the farm areas through dust and air particles. Livestock odours can be smelled as much as 5 or 6 miles away depending on factors such as weather conditions and farming techniques (Hribar and Schultz, 2010). Also, piggeries have dangerous levels of pollutants found in the air, in combination with dust particles. These contaminants consists of ammonia, carbon monoxide, hydrogen sulphide, particulate (inhalable and reparable dust), and endotoxins (Cole et al., 2000) that have an impact on the health and well-being of both animals and humans. Most studies focus on the health effects of the feeding operations air production on farm workers, however other studies have revealed the health effects of schools and children living in these environments (Hribar and Schultz, 2010). All neighbouring communities are at risk from poor air quality, but children take in 20-50% more air than adults, making them more susceptible to lung diseases and negative health effects (Hribar and Schultz, 2010). Hence, it is important to understand the characteristics of the gases emitted from pig waste or manure decomposition and their impacts on the environment, humans, and animals, so that farmers and researchers can precisely predict and research ways to control the odours emitted from livestock operations.

2.5 Greenhouse gas and volatile organic compounds emission

Besides lowering the air quality and water quality in the areas surrounding them, livestock operations also emit greenhouse gases that play a major role in climate change. Worldwide, approximately 18% of greenhouse gas is produced from livestock operations. All animal manures

(cattle, pig, chicken, sheep, and goats) are significant sources of greenhouse gases. The greenhouse gases related to animal management are carbon dioxide (CO_2), nitrous oxide (N_2O), hydrogen sulphide (H_2S), ammonia (NH_3), methane (CH_4), and other gaseous organic compounds which are collectively known as Volatile Organic Compounds (VOCs).

However, researchers have mainly focused on mitigating NH_3, CH_4, and H_2S while turning a blind eye to the emission of VOCs, which may cause odours and also contribute to greenhouse gas emissions (Amon et al., 2006b, Bastami, 2016). According to Oldenburg (1989), ammonia does not seem to be an important odorous compound, it was also reported that the mean ammonia concentrations were below 8 ppm in cattle barns, between 5 and 18 ppm in pig houses and between 5 and 30 ppm in poultry houses. Hydrogen sulphide is regarded as one of the most dangerous gases as it has been reported to be responsible for many animal and human deaths. However, its concentration is usually low, unless the manure is agitated (Le Phung et al., 2005).

Furthermore, NH_3, CH_4, and H_2S are all individual compounds, while odour is a complex mixture of various VOCs (Schiffman et al., 2001). More than 300 different odorous compounds may contribute to odour nuisance from livestock production facilities (Le Phung et al., 2005). Apart from Ammonia, VOCs organic compounds may be classified into three main groups: (1) sulphurous compounds, (2) indolic and phenolic compounds, and (3) volatile fatty acids (VFA). Proteins and fermentable carbohydrates are the main precursors of the formation of these compounds in the digestive tract of animals; in urine and in manure (Le et al., 2005). Volatile organic compounds (VOCs) also have a direct effect on greenhouse gas emission and global warming by absorbing heat radiation from the ground, but their indirect contribution is more significant. They react with hydroxyl radicals (OH-) in the air, forming tropospheric ozone, which

is a greenhouse gas. When they reach the stratosphere, they react with OH-, forming water vapour, which is a greenhouse gas (de Gouw and Warneke, 2007).

Since one can count several hundreds of compounds that are categorized as VOCs, one can also easily imagine the diverse environmental and health effects caused by these compounds. They range from local annoying odour emissions to global effects such as greenhouse phenomena (Ojala et al., 2015). Furthermore, certain VOCs are even more dangerous as the utilisation of carcinogens are limited (Ojala et al., 2015). Hence, more research needs to be conducted on how to mitigate VOCs from livestock manure for greater benefits to the environment and the economy.

2.5.2 Indolic and phenolic compounds

Indole and skatole are indolic compounds while phenol, p-cresol, 3-methyl phenol (m-cresol), and 4-ethyl phenol are phenolic compounds. These two compound types are classified as the main compounds responsible for odour emission in a piggery (Schiffman et al., 2001, Ni et al., 2012). The nature of the smell of indole and phenol compounds progress from the aromatic smell of phenol to the stench of indole and the nauseating smell of skatole (Le Phung et al., 2005). Phenolic compounds such as P-cresol) and 4-ethylphenol result from the microbial degradation of AA L-tyrosine in the intestinal tract of animals and in manure storage. If a pig is fed a protein rich diet consisting of L-tryptophan amino acid, Indoles are formed, partly absorbed and detoxified by the liver to glucuronides (i.e. 3-hydroxyindole, hydroxyskatoles and indole-3-carboxylic acid) (Moeser et al., 2001). Consequently, the indolic detoxification products are then excreted via urine. The unabsorbed part of indole and skatole is excreted via the faeces. Faeces contain a high level of b-glucuronidase of bacterial origin, which is an enzyme that hydrolyses glucuronides (Spoelstra, 1980, Le Phung et al., 2005). Hence, it is expected that mixing faeces with urine can cause an increase in free indolic compounds. During fermentation, the following bacteria are responsible

for forming indole from tryptophan*; E. coli* and *Proteus* (except *Proteus mirabilis*); some *Shigella; Aeromonas liquefaciens*; some *Fusobacterium* species; *Bacteroides melaninogenicus;* some *Bacteroides fragilis subspecies; Bacteroides coagulans; Paracolobactrum coliforme; Photobacterium harveyi; Bacillus alvei; some clostridia; Propionibacterium acnes; Micrococcus aero*genes (Le Phung et al., 2005, Shah and Gharbia, 2011). *E. coli,Citrobacter sp*., *Bacteroides fragilis subsp*. *thetaiotamicron*, *Clostridium* converts L-tryptophan to indole-3-acetic acid by the transamination of tryptophan to indolepyruvic acid and subsequent decarboxylation (Schiffman et al., 2001). Lactobacillus strains from the pig intestine then degrade indole-3-acetic acid to skatole (Le Phung et al., 2005).

Briefly, L-tryptophan results in the production of indole and skatole. There are three sources of indole and skatole compounds in manure: (1) degradation of the AA L-tryptophan in pig faeces; (2) direct excretion from the large intestine of the pig via faeces after being formed from tryptophan; (3) and emission from glucuronides in urine when placed in contact via faeces. Therefore, a better understanding of their sources, mechanism of production and types of emission will promote mitigation methods and technologies.

2.5.1 Sulphurous compounds

A number of authors have reported that sulphurous compounds are vital constituents of odour from livestock manure (Hanajima et al., 2010, Rincón et al., 2019, Rastogi et al., 2020). Sulphurous compounds are produced in two ways; (a) metabolism of sulphurous amino acids, and (b) sulphate reduction. The metabolism of sulphurous compounds occurs when manure is stored anaerobically. Organic sulphurous compounds such as the amino acids (AA) methionine, cysteine, and cystine are broken down to release sulfidic compounds (Razote et al., 2004). These sulphurous AA are used as a source of carbon and energy by microbes in manure during fermentation. These processes

are performed by various anaerobic bacteria in the faeces which results in the production of some intermediates that can volatilise and create odour. A typical example of this is the hydrolysis of methionine, which forms methanethiol (methyl mercaptan) and is further degraded to sulphide. As a product of L-methionine, Methanethiol is then chemically converted to dimethyl disulphide and dimethyl trisulphide in the presence of Cu(II) or ascorbate plus Fe(III) (Le Phung et al., 2005). Sulphide reduction is the main source of sulphate in animal manure. This process is either as assimilatory or dissimilatory and it involves bacteria that are sulphate reducers which belong to the species; *Desulfonema*, *Desulfovibrio*, *Desulfotomaculum*, *Desulfobacter* and *Desulfococcu* (Spoelstra, 1980). In the assimilatory process, bacteria produces enough reduced Sulphur for the biosynthesis of cysteine and methionine, unlike in the dissimilatory process were sulphate is used as an electron acceptor for an anaerobic respiration, which leads to a high amount of odour being produced (Schaefer and Bemelnans, 1974, Le Phung et al., 2005).

Moreover, it has been reported by Le Phung et al. (2005) that sulphurous compounds are the most offensive compounds, the odorous nature of this compound progresses from the putrid smell of dimethyl disulphide and methanethiol to the rotten eggs smell of hydrogen sulphide. It has also been reported that apart from hydrogen sulphide, methanethiol and dimethyl disulphide may be very important odorous compounds to be considered. However, research has focused only on hydrogen sulphide. Besides hydrogen sulphide, other sulphurous compounds identified from livestock production facilities include; carbon disulphide, 2-propanethiol, dimethyl disulphide, dimethyl trisulphide, 2-methylthiopropane and dimethyl hexasulphide (Oehrl et al., 2001, Ni et al., 2012). More research needs to be conducted on how to mitigate these compounds emissions influenced by diet and animal type.

2.5.3 Volatile fatty acids (VFAs)

Volatile fatty acids are mostly formed by microbial conversions of plant fibre and protein residues in the large intestine and in manure under anaerobic conditions (Owens and Basalan, 2016). Plant fibre residues, such as lignin, are very difficult to break-down under anaerobic conditions. Cellulose and hemicellulose are first hydrolysed by microbial enzymes into oligomers and/or monomers. The latter are subsequently converted by the microbes into volatile fatty acids such as acetic, propionic, valeric acid, and butyric acids isomers (Le Phung et al., 2005). In animal production facilities, volatile fatty acids are commonly reported as one of the major constituents of gaseous emissions. About 60% of the total VFA in manure (w/w) are present as acetic acid (Spoelstra, 1980, Oehrl et al., 2001). This is because animal waste is richer in fibre, and fibre fermentation is often dominant in acetic acid proportion. Acetic, propionic and butyric acids are also formed by the deamination of amino acids (AA) such as; L-glutamate, L-lysine, and L-alanine. Ammonia, CO2 and [H] are additional end products of this deamination–decarboxylation (Le Phung et al., 2005). Peptolytic bacteria hydrolyse proteins into AA which can later be deaminated and decarboxylated to branched-chain VFA (Spoelstra, 1980). In the gastrointestinal tract of pigs (hindgut), these short-chain VFA (fatty acids with chain lengths of two to six C atoms) can be eliminated as waste when not absorbed, hence the presence of volatile emissions such as VFAs from animal litter (Razote et al., 2004).

Volatile fatty acids are produced from carbohydrates and sometimes proteins under anaerobic conditions in the large intestine of animals and in manure storage. Carbohydrates are transformed to straight-chain VFA only. Proteins are transformed to both straight-chain VFA and branched chain VFA. Short-chain VFA in the large intestine can be used as an energy source for the host animal and might not be a big problem in terms of odour nuisance (Spoelstra, 1980, Le Phung et

al., 2005, Bibbiani and Russo, 2012). However, when they are in manure storages, VFA may be volatilised and cause malodour. Therefore, there is a need to continue to understand the different VFAs produced, and to explore strategies on how to mitigate emissions from manure. For better understanding of practical odour mitigation systems, reducing odour production from both manure and animal diet could be important. Feed additives such as effective microorganism (EM) is one of the biochemical and chemical agents that can be used to reduce odour from animal production. However, their use to reduce odorous compounds emitted from livestock manure has not been well explored. The principles of using feed additives such as EM to mitigate odour formation and emissions are; to change the microflora in the large intestine or the manure, and to change the pH into a less favourable one for odour producing microbes. This shows that effective micro-organisms can play a vital role in odour mitigation, hence the need to review effective micro-organisms types, characteristics, as well as functional mechanisms and emissions mitigation mechanisms.

2.8 Effective microorganism (EM)

Effective microorganism (EM) is a mixture of cultured microbes and naturally occurring microorganisms that are beneficial to both animals and their environment. It is composed mainly of photosynthetic bacteria, actinomycetes, yeast, lactobacillus, and fungi (Mafiri, 2014). This technology was developed during the 1970s in Japan (Freitag and Meihoefer, 2000). In 1982, it was discovered that certain mixtures of biochemical functions was promoted when blended in a state resulting in healthy plant growth and a more abundant harvest of better testing crops (Freitag and Meihoefer, 2000). Later in the mid 1980's, livestock researchers and producers began to test EM for odour control and waste management. It was discovered that EM could be an effective tool for waste treatment and odour control, which is the most valuable contribution in the livestock

industry (Kitazato Environmental Science Center 1994). Effective microorganisms has spread extensively to 150 countries around the world and is now a global technology used to improve soil quality, enhance crop and animal production, reduce odour emissions, protect, conserve natural resource, and to create more sustainable agriculture and environment (Wing and Wolf, 2000). However, its functions in controlling odour emission are very limited in the literature, hence there is a need to study its biochemical functions.

2.8.1 Biochemical functions of effective microorganisms

Effective microorganisms consist mainly of 5 components; photosynthetic bacteria, lactic acid bacteria, yeast, actinomycetes and fermenting fungi. Each component has its own important function. Photosynthetic bacteria are independent, self-supporting microorganisms, and pivot to effective microorganism activities. These bacteria use sunlight as an energy source to synthesize useful substances, such as amino acids, nucleic acid, sugars and bioactive substances to promote plant growth and development from secretions of roots, organic matter and harmful gases (Esatu et al., 2011), this event is termed "coexistence and co-prosperity." Lactic acid bacteria is a strong sterilizer (pH between 3.4 and 3.7), which suppresses harmful microorganisms and increase rapid decomposition of organic matter such as lignin and cellulose without causing any harmful influences occurring from undecomposed organic matter (Mafiri, 2014). Yeast produces antimicrobial substances from sugars and amino acids that are produced by photosynthetic bacteria and organic matter. These substances kill all harmful pathogens, and therefore the majority of pathogens cannot survive in the effective microorganism medium. Yeast also produces beneficial substances, such as hormones, enzymes and vitamin B (Esatu et al., 2011). Actinomycetes co-exist with photosynthetic bacteria to produce antimicrobial substances, which suppress the growth of harmful fungi and bacteria (Esatu et al., 2011). Finally, some fungi such as aspergillus and

penicillium rapidly produce alcohol, esters and antimicrobial substances by decomposing organic matter. Hence, suppressing the odours usually produced by organic matter or waste fermentation. This process is said to prevent infestation of harmful insects and maggots (Mafiri, 2014).

2.8.2 Use of effective microorganism for odour control from animal manure or slurry

The use of effective microorganism to reduce odour in the environment is not well researched. High concentration of VOCs can be hazardous to the environment, humans and animals. About 10-12% of the world's total greenhouse emission comes from agriculture, while manure management alone is said to be responsible for 13% of the greenhouse emission from the agricultural sector (Brouček and Čermák, 2015). Various complaints from residents living nearby livestock operations have increased over the years. People complain about the health impacts posed by gas emissions and the decrease of the value of properties due to the odours that drive away customers from hotels and restaurants. An optional strategy to reduce gaseous emissions from livestock manure is needed immediately with such high numbers of complaints and the potential health risks. This is a significant challenge for the livestock industry to produce healthy livestock products, while meeting the increasing demand for livestock products and simultaneously reducing the nuisance of odours. Various strategies to reduce odour from livestock manure have been proposed such as separating the solid and liquid fractions of cattle slurry, but this may result in total manure N loss in the separated solid fraction (Smith et al., 2014). This also requires enhanced infrastructure, management and maintenance cost to the farming business, which may not be favoured by a typical small-scale farmer. Mpendulo (2012) conducted an experiment where they added high fibre ingredients (such as lurcen hay, sunflower husk and maize cob) in pig rations to measure its effect on odours emitted from pig slurry tanks. The results were unclear and inconclusive on the emissions hence more work still needs to be done to characterize

compounds from the large variety of locally available fibre sources that reduce water and air pollution from pig enterprises. Amon et al. (2006a) reported that the application of EM in cattle slurries relatively reduced the emissions of NH_3 and N_2O, but slightly increased CH_4. This showed the potential of EM as an agent to reduce NH_3 and greenhouse gas emissions from stored slurry even though volatile organic compounds were not considered. Bastami (2016) obtained cattle manure or slurry from a commercial farm in two different seasons (winter and summer) and treated it with a mixture of actiferm EM and glucose at different levels and reported a decrease in greenhouse gases (CH_4, CO_2, and N_2O) emissions during 120 days of winter compared to summer storage. However, VOCs emissions or reduction were not observed. There is a need to evaluate the full potential of the use of EM in the reduction of all the harmful gases that can be emitted from animal manure. The use of EM in treatment of animal manure, particularly swine and beef cattle is not well explored. There are only a few papers about odour emission from feedlot cattle and most of the research was carried out in Europe and North America (Mielcarek and Rzeznik, 2015). Fewer studies on manure storage and treatment units or open feedlots (Casey et al., 2006) were available in the literature. Consequently, there is a need to evaluate the role of effective microorganisms in swine and beef cattle performance and control of odour emission.

2.9 Summary

Animal nutritionists have been ignoring odour emissions when formulating livestock feeds due to increasing pressure to meet the demand of fast animal growth to supply animal protein for humans. The impact of these high quality diets on animal waste is critical as it may play a role in terms of environmental pollution. These diets have the potential to either increase or decrease gaseous emissions which can be a problem to the community surrounding the source. The impact of odour on workers, local communities and the environment cannot be ignored anymore as it has the

potential to limit the expansion of livestock production enterprises (Mpendulo, 2012). This poses a threat to the livestock production enterprises since some of them have been forced to shut down due to complaints from local residents while other enterprises are facing lawsuits because of the bad odour emitted by manure on farms. Livestock production is considered to be financially, nutritionally and culturally important to the people of South Africa (Mafiri, 2014). There is limited information on the treatment of livestock manure, particularly swine and feedlot cattle for the reduction of VOCs especially analysis of potential toxic gases that can be emitted. Therefore, there is a need to research cost-effective solutions that can reduce odour emissions and protect the health of both animals and farm workers as well as community members. Good results may prevent feedlots or other farms from shutting down or moving too far from consumers which often have a major cost implication. Global warming and diseases in both human and livestock is aggravated by air pollution from livestock waste which is currently receiving more attention as waste management is a growing research in South Africa advanced by the Government (Council, 2003, Kleinschmit, 2009, Archer van Garderen, 2011).

CHAPTER THREE: Effect of feed type and effective microorganisms on feedlot cattle performance and litter odour emission, respectively.

Abstract

One of the major constraints to the development of more efficient and sustainable beef production is the generation of odour from manure, rearing, handling and storage facilities. The gaseous emissions could be odorous or odourless and may have some toxic potential on both human and livestock performance. Various strategies to mitigate odour from beef manure have been suggested but this chapter investigates the effect of feed type and effective microorganisms on feedlot cattle performance and litter odour emission, respectively. A total of 50 bonsmara beef cattle were used in the study. They were housed in feedlot pens and fed on various diets (Starter, grower and finisher) at different stages of growth. The diets formulated on farm consisted of maize, lucerne hay, silage, molasses and HPC (high protein concentrate). The manure (faeces) was collected from the beef feedlot randomly from 20 different faecal deposits to make sixteen samples of 300g and treated with EM at different concentrations (0% EM (Control); 10% EM; 20% EM; and 30% EM). Chemical composition of feed was determined for feed quality, feed intake, and weight gain and feed conversion ratio calculated for animal performance and gaseous emissions were trapped from different litter and determined using a coupled Varian 3800 gas chromatograph (GC). The results obtained from this study indicated that diet improved animal performance with increased ($P<0.05$) feed intake, weight gain and feed conversion ratio. The test for differences in odour emission using feed type as a factor (Across all week groups) indicated that diet had an effect on odour emission since grower manure emitted high ($P<0.05$) amounts of gases compared to starter manure. The gases emitted from the manure of different diets were classified into: sulphurous compounds, and phenols volatile fatty acids (VFAs). Acetic acid and phenol were identified as the dominant toxic

compounds to human kind. Dimethyl sulphide and dimethyl trisulphide were detected as the main dominant odour causing compounds in cattle. All these gases were successfully reduced ($P<0.05$) by EM except for dimethyl trisulphide. Furthermore, the use of EM at different treatment levels to mitigate odour emission indicated that there was no significant difference across all treatment levels ($P>0.05$) but for the control. It was then noted that EM does not depend on concentration to reduce odour from litter but depends on time for the microbes to adapt and compete with the microbes in the manure since there was a reduction ($P<0.05$) of gases emitted on the different treatment groups compared to the control. A 10% dose of EM was recommended for cattle manure treatment to reduce gaseous emissions since higher doses effect was minimal.

Keywords: Effective microorganisms, odour, volatile organic compounds, grower, starter, acetic acid, phenol, dimethyl sulphide, dimethyl trisulphide.

3.1 Introduction

Beef cattle production has evolved over the last few decades and shifted from integrated farms to intensive systems using confined facilities (Bibbiani and Russo, 2012). To fulfil consumer demand for meat and milk products, there has been an increase in cattle numbers reared in intensive farms over the past decade (Chung et al., 2013). A large number of animals reared in a small area results in production of large quantities of excreta (faeces and urine). Hence, manure management has become more important from both agronomic and environmental perspectives. Cattle manure is a significant source of VOCs emission. Therefore, different strategies have been explored and implemented to reduce emissions, e.g. production of biogas, which also (primarily) generates renewable energy (Bastami, 2016), regular cleaning and drying up of manure, fertilization of pasture and rare cases used by small scale farmers for building walls. However, two important factors need to be considered when planning and building biogas biofuel facilities; (a) capital and

maintenance costs; and (b) the number of livestock needed to make the system economically viable and productive (Conti et al., 2019). These factors can be significant barriers to small-medium farmers, especially in South African cattle farming systems. Inefficient and impractical biogas production approaches for the small-medium farms size means that there is a need for alternative approaches to reduce VOCs emissions from cattle manure. Volatile organic compounds (VOCs) which represent odour, are mainly generated by microbial conversions of non-utilized dietary nutrients and endogenous products excreted in the faeces under anaerobic conditions (Le Phung et al., 2005). Therefore, if the process can be slowed down or stopped, microbial fermentation of organic matter in manure before the hydrolytic and acetogenic bacteria become active, can reduce or prevent the formation of VOCs. However, very few studies so far have focused on reducing the formation of odorous compounds at source, for example, in the large intestine of the animal or in manure storage (Le Phung et al., 2005).

The use of microbial substances or organisms such as effective microorganism (EM) either as a mixture or single culture, e.g. *Lactobacillus plantarum*, or *Lactococus lactis* is a possible approach to reduce VOCs. However, the effectiveness of a single culture or species remains unconvincing, as cattle manure contains multiple dominant microorganisms responsible for fermentation (Bastami, 2016), which contributes to the total VOCs emission. The use of EM could inhibit the fermentation and the odour produced which implies that nutrients would be retained. This can only be realized by destroying the pathogens which are responsible for gaseous emissions. Some studies have shown signs of decrease in gaseous emission while paying particular attention to CH4, NH3 and CO2. There is need to expand the research by investigating all the other emissions apart from the previous three mentioned. Hence, the aim of this study was to provide an initial evaluation on

how different diet fed to beef cattle will contribute to gaseous emissions and the potential of EM to mitigate these emissions from beef manure.

3.2 Materials and Methods

3.2.1 Study site

The Feeding study was conducted at Triple A farm Pietermaritzburg, KwaZulu-Natal, South Africa. Triple A farm is located within the latitude of -29.6168 and longitude of 30.3928, with an altitude of 764.7432m. The daily temperatures average 29°C, with variation ranging from 28.2 to 43°C (SA Explorer, 2014). Laboratory analysis of gas emissions and chemical composition of feed was conducted at the University of Zululand, KwaDlangezwa campus in KwaZulu-Natal Province of South Africa. The University of Zululand is located within the latitude of -28°51'16" and a longitude of 31°50'45", with an altitude of approximately 121m. The climate is subtropical with average temperatures of 28.4°C and 14.5°C in the summer and winter respectively. The average rainfall varies from 670mm to 970mm per year experiencing summer rainfalls from October to March (SA Explorer, 2014).

3.2.2 Experimental animals and housing

A total of 50 bonsmara beef cattle (25 heifers and 25 bulls) were used in this study. Beef feedlot animals were sourced from triple A. The animals were of similar age and were received on the same day and in the same pen. Cattle pens were made up of concrete floors and provided enough shade. Feed hoppers were designed to avoid spillage and facilitate the collection of residues. Clean water was provided ad libitum. Pens were designed in such a way that faeces and urine were collected separately.

3.2.3 Animal Feeding and care

The feedlot animals from triple A were received in the welcoming pen with sufficient shade provided with hay and water ad lib for three days before sorting. After vaccinating, weighing and tagging 50 animals were sorted into a pen where they were fed for the first three weeks with a starter ration. Grower was the next feed provided for 3 weeks and finisher was the last feed which was provided for 2 weeks. The animals were fed thrice a day with feed consisting of ingredients such as maize, lucerne hay, silage, molasses and HPC (high protein concentrate) during the different stages of growth.

3.2.4 Chemical composition of feeds

3.2.4.1 Dry Matter and moisture content of feed

Dry matter of the feeds were determined according to the AOAC (2002) method (AOAC Official Method Number 934.01). Clean petri dishes were dried in the oven at 105°C for 2h, cooled in a desiccator and weighed (W_0). Approximately a 1 gram sample was transferred into a petri dish and weighed again (W_1). The dishes with samples were oven dried at 60ºC overnight and cooled in a desiccator before weighing (W_2). The dry matter content was then calculated as the percentage per gram sample.

$$\%\text{Dry Matter} = \frac{W1-W2}{W1-W0} X100$$

3.2.4.2 Ash content

Ash content was determined according to AOAC International (2002), AOAC Official Method 942.05. Approximately 1 g of dried feed sample was weighed into a crucible and placed in the muffle furnace at 100ºC for 12 hours. At the end of the ash-ing period, the furnace was turned off and allowed to cool for 20-22 hours. The samples were removed from the furnace with tongs and

cooled in a desiccator before weighing. That weight was the gross ash weight and the sample ash weight was determined by subtracting the crucible weight difference from the gross ash weight. The percent ash was expressed as the ratio of the sample ash weight to the dry sample weight multiplied by 100. Ash was expressed as;

$$\text{Ash} = \frac{weight\ after}{weight\ before}\ X\ 100$$

3.2.5 Fibre content of feed

3.2.5.1 Neutral detergent fiber (NDF)

Neutral detergent fibre was determined as described by Van Soest et al. (1991) using the ANKOM 220 fibre analyser (Ankom Technology, Fairport, New York). Approximately 1 g sample (W_1) was weighed into F57 ANKOM filter bags. The samples were refluxed for 60 min in 50 ml neutral detergent fibre solution (30g sodiumdodecyl sulphate, 18.61g ethylenediaminetetraacetic sodium salt, 6.81g sodium borate, 4.56g sodium phosphate dibasic in 1L distilled water). After refluxing, the reagent was filtered off with the aid of a vacuum suction and the samples were rinsed 3 times in boiled water and placed in acetone for 3 minutes before air drying for 40 minutes. The air-dried samples were dried overnight at 60ºC and cooled down in a desiccator for 30 minutes before weighing (W_2) at room temperature to determine NDF content.

$$\%\text{NDF} = \frac{Weight\ of\ residue\ (W2 - W1)}{Weight\ of\ sample\ (W1)} X100$$

3.2.5.2 Acid detergent fiber

Neutral detergent fibre (NDF) residue was used to determine acid detergent fibre (ADF) as described by Van Soest et al. (1991) using the ANKOM 220 fibre analyser (Ankom Technology, Fairport, New York). The NDF residues (W_1) were refluxed for 60 min in 50 ml acid detergent fibre solution (20 g cetyl trimethylammonium bromide to 1L 1.0N H2SO4). After refluxing, the

reagent was filtered off with the aid of a vacuum suction and the samples were rinsed 3 times in boiled water, then placed in acetone for 3 minutes before air-drying for 40 minutes. The samples were then oven dried at 60°C overnight and weighed (W_2). The acid detergent fibre was calculated using the formula below;

$$\%ADF= \frac{Weight\ of\ residue\ (W2 - W1)}{Weight\ of\ sample\ (W1)} X100$$

3.2.5.4 Crude Protein (CP)

Crude Protein was determined according to the Kjeldahl method (Horwitz, 2000), AOAC Official Method Number 954.01. Approximately 1 g of sample with a 10 g mixture potassium sulphate and copper sulphate as the catalyst will be digested in 25 ml concentrated sulphuric acid at 420°C. After digestion, cooled samples were diluted with 75 ml distilled water and 1% boric acid and titrated again with 0.5 M hydrochloric acid. The indicators bromocresol green and methyl red were used. The percentage of total nitrogen and of crude protein was calculated using the following formulas:

$$\%\text{Total Nitrogen} = \frac{(14.01\ X\ M\ X\ 100)}{g\ Sample} X\ (ml\ titrant - ml\ blank)$$

% Crude Protein = N x 6.25

3.2.5.5 Non-polar extracts (NPE)

Hemi-cellulose was determined according to Van Soest et al. (1991) method by subtracting ADF from NDF.

3.2.6 Growth and performance of Pig and feedlot cattle

3.2.6.1 Feed intake

The amount of feed consumed by animals were measured every day. The amount of feed was measured in grams per cattle. Feed intake in each replicate was measured using the following formula:

Feed intake (FI) = feed provided (kg) – feed left over (kg)

3.2.6.2 Weight gain

The animals' weight were measured every time the animals' moved from one feeding ration to another to monitor their performance. The overall body weight gained was determined between by subtracting the initial body weight from the final body weight.

3.2.6.3 Feed conversion ratio

Feed conversion ratio (FCR) is a relationship between feed consumed and muscle deposition.

The FCR was measured every time the animal moved from one feeding stage to another with the formula:

$$FCR = \frac{\text{Total quantity of feed consumed per animal in kg}}{\text{Average body weight}}$$

3.2.7 Preparation of effective microorganism

3.2.7.1 Effective microorganism solution

Effective microorganism solution was obtained from Efficient Microbes in Durban, South Africa. The solution is called Super EM which is a water-soluble liquid concentration of probiotic microorganisms (Probio, 2016). Super EM is an inactive solution, which can be activated by adding water. The EM was activated by adding water in different concentrations (EM0%, EM10%;

EM20% and EM30%,) before using to spray the cattle faeces for odour control. Each treatment had three (3) replicates for accuracy purposes.

3.2.8 Faecal sampling

3.2.8.1 Preparation of samples for odour measurements

Faecal samples for treatment with EM were collected from the starter, grower and finisher pens. Fresh faecal samples were as collected randomly from 20 different faecal deposits in the starter pen mixed thoroughly before using to make 16 samples of 300g each in a 1L glass beaker (79mm x 39.5mm radius). These samples were used to form four groups of four replicates for treatment with EM. The activated EM solution at different concentrations (0% EM (Control); 10% EM; 20% EM; and 30% EM) were used to spray the different groups. The same faecal procedure and treatment was repeated when the animals moved to the grower and finisher pens. The height of the samples (20cm) in the beaker was similar to the height of cattle faecal deposits as observed in the feedlot natural environment. The samples were treated with EM by spraying at different concentrations and the gaseous emission were captured in traps (odour measured) once a week for four weeks. The samples were kept in a well-ventilated room and 2 m apart at the university farm house during experimentation.

3.2.9 Odour sampling

For odour sampling, each glass beaker was placed into special polyacetate bags (Nalo Bratfolie Kalle GmbH- Germany) and sealed prior to sampling (in order to concentrate the volatile gases or compound for about 10min) with an adsorbent trap (not in contact with faeces) connected by a tube to a portable battery operated pump outside (Spectrex Personal Air Sampler PAS 500, United States of America). The pumps calibrated at 200ml were used to suck air into the absorbent traps for 30min. Air samples were simultaneously collected from empty polyacetate bags placed away

from the feedlot as controls to identify background contamination. The traps were stored at -20°C in a sealed vial until analysis. The traps contain 2mg of a 50:50 mixture of Tenax TA (Alltech Associates, USA) and graphitized carbon (Carbotrap TM, Supelco, USA) in a glass tube closed at both ends with glass wool. The gaseous emissions were collected every week before treatment with EM.. The stored traps were analysed using a coupled Varian 3800 gas chromatograph-mass spectrometer (GC-MS).

3.2.10 Analysis of gaseous emissions and compound identification

Volatile samples were analysed using a coupled Varian 3800 gas chromatograph (Varian Palo Alto, CA, USA) and Varian 1200 mass spectrometer. The gas chromatograph was equipped with an Alltech EC–WAX column of 30 m × 0.32 mm internal diameter × 0.25 mm film thickness (Alltech Associates Inc.,Deerfield, IL, USA). Helium was used as the carrier gas at a flow rate of 1 mL min^{-1}. The traps were placed in a Varian 1079 injector by means of a Chromatoprobe fitting and thermally desorbed by heating the injector at 40 °C for 2 min with a 20:1 split ratio and then increased to 200 °C, and then held at 200 °C min^{-1} in splitless mode for thermal desorption. After a 3 min hold at 40 °C, the gas chromatograph oven was ramped up to 240 °C at 10 °C min^{-1} and held there for 12 min. Compound identification was carried out using the Varian Workstation software with NIST05 mass spectral library and comparisons with retention times of chemical standards, where available, as well as comparisons between calculated Kovats retention indices and those published in the literature. A homologous series of alkanes (C8–C20) was used to determine Kovats retention indices. Compounds were verified using retention times of authentic standards (97–99.5% Sigma Aldrich Inc. GmbH, Germany), and (3E)-1, 3-octadiene (98 %, ChemSampco, USA), and published Kovats indices. Compounds present at higher or similar percentages in controls were considered as contaminants and excluded from the analysis. For

quantification of emission rates, known amounts of standards of dominant compounds were injected into cartridges and thermally desorbed under identical conditions to the samples. The peak areas of compounds in the samples were compared with those of the standards and used to calculate the total emission rates of compounds per faecal sample per hour, and emission rate per compound per faecal sample per hour. Individual compounds comprising ≥10% of the average relative amount that were emitted in almost all growth stages and during different feeding rations were considered dominant compounds.

3.2.11 Statistical analysis

All data were analysed using statistical package of social science (SPSS). The means of chemical components of the different diets (DM, OM, Ash, Fat, MC, NDF, ADF, HEM, CP, Ca, Mg, K, Na; P, Zn, Cu, Mn, and Fe) and performance parameters (weight gain, feed conversion ratio, and feed intake) were analysed using analysis of variance (ANOVA) under the statistical package of social science. Tukey's test was used for comparison between means of chemical components and performance parameters which were different when $P<0.05$.

For odour analysis, Primer 6 program (Clarke and Gorley, 2006) was used to analyse and compare scent profiles emitted from manure of beef fed with different diets (starter, grower, finisher). Nonmetricmultidimensional scaling (NMDS), based on Bray–Curtis similarities of square root transformed data, was used to detect similarities among samples. To evaluate how well or poorly the particular configuration reproduces the observed distance matrix, the stress value is given. The smaller the stress value, the better the fit of the reproduced ordination to the observed distance matrix (Clarke, 1993). Differences in scent profiles between different feeding stages was assessed by ANOSIM (Clarke and Gorley, 2006) with 10 000 random permutations.

3.3 Results

3.3.1 Chemical composition of feeds

There were significant differences (P<0.05) in all feed components measured apart from magnesium, K/Ca+Mg, and copper (Table 4.1). Grower had the highest (P<0.05) dry matter, fibre (NDF, ADF and hemicellulose) compared to starter and finisher while its crude protein content was the same with starter but higher than in the finisher diet. The feedlot starter ration was observed with the highest (P<0.05) moisture content in comparison with other diets.

Table 3.1 Chemical composition of diet provided to beef cattle

Components	**Feed type**				
	Starter	**Grower**	**Finisher**	**SEM**	**P-value**
DM (%)	70.63^{a}	76.22^{c}	71.66^{b}	1.09	0.05
MC (%)	29.22	23.93	28.14	1.09	0.05
Ash (%)	8.48^{b}	7.99^{b}	6.75^{a}	0.31	0.05
Fat (%)	4.84^{a}	4.71^{a}	6.58^{b}	0.37	0.05
OM (%)	91.53^{a}	92.01^{a}	93.26^{b}	0.33	0.05
NDF (%)	25.57^{b}	26.86^{c}	19.54^{a}	1.43	0.05
ADF (%)	7.76^{a}	10.88^{b}	7.52^{a}	0.70	0.05
HEM(%)	17.81^{c}	15.98^{b}	12.02^{a}	1.09	0.05
CP (%)	13.78^{b}	14.28^{b}	11.89^{a}	0.45	0.05
Ca (%)	1.26^{b}	0.99^{ab}	0.85^{a}	0.08	0.05
Mg (%)	0.37^{a}	0.36^{a}	0.29^{a}	0.17	N
K (%)	1.35^{b}	1.36^{b}	1.18^{a}	0.04	0.05
Na (%)	0.14^{a}	0.15^{a}	0.21^{b}	0.17	0.05
K/Ca+Mg(%)	0.37^{a}	0.44^{a}	0.46^{a}	0.02	N
P (%)	0.39^{b}	0.38^{b}	0.29^{a}	0.02	0.05
Zn (ppm)	121.56^{b}	108.11^{b}	94.72^{a}	4.97	0.05
Cu (ppm)	14.68^{a}	14.38^{a}	15.58^{a}	0.47	N
Mn (ppm)	69.97^{b}	76.14^{b}	50.26^{a}	5.25	0.05
Fe (ppm)	1121.05^{b}	1126.35^{b}	821.40^{a}	65.66	0.05

DM= Dry matter; MC= Moisture content; OM= Organic matter; NDF=Nutrient detergent fiber; ADF= Acid detergent fiber; HEM= Hemicellulose; CP= Crude protein; Ca= Calcium; Mg= Magnesium; K= Potassium; Na= Sodium; P= Phosphorus; Zn= Zinc; Cu= Copper; Mn= Manganese; Fe= Iron, SEM= Standard error of means; N= No significant difference. Means within the same raw with different subscripts are significantly different (P<0.05).

3.3.2 Growth and performance of feedlot cattle

There was a significant difference (P<0.05) across all the performance parameters (intake, weight gained and FCR) measured when animals were fed different types of diets (Table 3.2). The highest (P<0.05) feed intake and feed conversion ratio was observed when animals were fed with finisher feed ration. Animals fed with grower had high (P<0.05) weight gain compared to the other diets. Feed intake and feed conversion ratio was observed to increase as the animal changes feed from starter diet to finisher.

Table 3.2 Beef growth and performance

Components	**Feed type**				
	Starter	**Grower**	**Finisher**	**SEM**	**P-value**
Feed intake (Kg/cattle/day)	4.05^{a}	9.10^{b}	10.05^{c}	1.18	0.05
Weight gain (Kg/cattle/day)	0.62^{a}	1.42^{b}	1.39^{b}	0.17	0.05
Feed conversion ratio	0.25^{a}	6.41^{b}	7.21^{c}	1.44	0.05

SEM; Standard error of means. Means within the same raw with different subscripts are significantly different (P<0.05).

3.3.3 Effect of EM on gaseous emissions from manure of beef cattle fed with starter diet.

The volatiles emitted by the faecal samples from beef cattle fed starter rations are summarized in Table 3.3a, b, c, d and e. The compounds were identified by their common names and CAS (Chemical Abstract Service) registry numbers and listed according to estimated Kovats retention index (KRI). In total, 44 compounds were identified, which included 4 alcohols, 6 aldehydes, 13 volatile fatty acids (VFA), 3 ketones, 8 Phenolics, 2 Terpenoids, 3 Amides, 2 Sulphur compounds and 3 nitrogen containing compounds. In the first week after fermentation and treatment of faecal samples with EM at different concentrations [ST0 (control); ST1 (10% EM); ST2 (20% EM) and ST3 (30% EM)], a total of 42 compounds were identified. 1-hexanol and Decanal were the only alcohol and aldehyde compounds emitted in high amounts but were mitigated with treatment ST2.

A total of 18 VFAs were emitted, with the dominance of acetic acid, butanoic acid and isocrotonic acid. These compounds were reduced ($P<0.05$) with ST3 except for butanoic acid which slightly increased by 0.02%. Phenol and 3-methyl phenol were the most dominant phenolic compounds but it reduced by ST3. Only one sulphur compound was emitted, which was dimethyl sulfone and it was highly mitigated at ST1.

After week 2, 43 compounds were detected. All of these compounds were mitigated ($P<0.05$) somehow by ST3. Decanal, acetic acid, propanoic acid, isocrotonic acid and phenol were further mitigated into lower concentrations by treatment ST3 compared to week 1. Furthermore, in week 3 only 34 volatile compounds were identified (Table 3.3b). All compounds in treatment ST3 were in lower ($P<0.05$) concentration and some were even not detected, except for acetic acid which was in higher concentration than all the compounds. 1-hexanol, butanoic acid, isocrotonic acid, dimethyl sulfone, Acetamide, N-methyl (Methylacetamide) and butanamide were all completely mitigated to 0% in ST3 and were only detected in the control (ST0), ST1 and ST2. Phenol, and 3-methyl-phenol were in low concentration in ST3 but higher in the control.

3.3.4 Effect of EM on gaseous emissions from manure of beef cattle fed with grower diet.

A total of 46 compounds were identified, comprising of 4 alcohols, 6 aldehydes, 14 volatile fatty acids (VFA), 4 ketones, 7 Phenolics, 5 Terpenoids, 1 Amide, 3 Sulphur compounds and 2 nitrogen containing compounds (Table 3.3a,b,c,d and e). These results showed that there were less ($P<0.05$) compounds in manure emitted by cattle fed grower than starter. This differences were accounted for by the presence of more ketones, less phenolic, more terpernoids, less amides and more Sulphur compounds.

Out of 39 compounds emitted in week 1, Nonanal, Butanoic acid, Benzyl alcohol, and Phenol were the most dominant compounds emitted but were decreased to undetectable concentrations by

treatment GT3. Phenol increased ($P<0.05$) to higher concentrations in GT3 compared to the control. Sulphur compounds (dimethyl disulphide, dimethyl trisulphide) were completely mitigated to 0% with GT3 treatment. Moreover, there were no nitrogen compounds emitted in the first week although they started appearing on the 2nd week during fermentation.

In week 2, 35 compounds were identified, 1-butanol; nonanal, acetic acid and pentanoic acid were the emitted in high concentrations at GT0 but were reduced ($P<0.05$) with treatment GT3. There was still no reduction of phenol in treatment GT3 but interestingly, it was absent in treatment GT1. A total of 36 compounds were emitted in week 3, this included the dominance of nonanal, which was higher in the control but decreased in treatment GT3. There were no alcohols emitted except for 1-butanol which was in high concentration in treatment GT1 and GT3. Phenol were finally mitigated to low concentrations in treatment GT3. Pyridine, 2,3,6-trimethyl and 2-Pyrrolidinone were the most dominant nitrogen compounds which were higher in the control but they were completely mitigated to 0% with treatment GT2.

3.3.5 Effect of EM on gaseous emissions from manure of beef cattle fed with finisher diet.

The gaseous compounds emitted were 50 in total, these consisted of 4 alcohols, 6 aldehydes, 15 volatile fatty acids (VFA), 3 ketones, 9 Phenolics, 4 Terpenoids, 3 Amides, 3 Sulphur compounds and 3 nitrogen compounds (Table 3.5a, b, c, d & e). A total of 46 compounds were detected after week 1. Heptanal, Octanal and Nonanoic acid were observed at lower ($P<0.05$) concentrations in the control but increased with treatment FT1 before decreasing with treatment FT3. Butanoic acid was the dominant VFA which was emitted in higher concentration yet it was mitigated to low concentration with treatment FT3. Phenol, Dimethyl trisulphide, Dimethyl sulfone and Indole were the most dominant odour causing compounds and were decreased to undetectable concentrations with treatment FT3. Phenol somehow increased with treatment FT3.

During week 2 the number of compounds emitted decreased to 40, this included the complete mitigation of odorous compounds such as butanamide, dimethyl sulfone and indole. The most dominant compounds emitted in higher amounts in the control but decreased with treatment FT3 were; 1-butanol; nonanal; and butanoic acid. Instead of decreasing, dimethyl disulphide was observed to increase high concentration in treatment FT3. In the 3rd week, dimethyl disulphide was observed to highly decrease with treatment FT3. A further decrease of the number and concentration of compounds emitted was observed in week 3. A total of 36 compounds were identified, no ketones and amide compounds were detected accept in the control. Butanoic acid, phenol, 3-ethyl phenol, butanamide, dimethyl trisulphide, and dimethyl sulfone were the most dominant compounds that were completely mitigated by treatment FT3 but were only detected in the control. 1-butanol and 2-Methylhexanoic acid did not decrease with treatment FT3.

3.3.6 Summary statistics and level of significance for odour emission

Overall, a two way ANOSIM tests for differences in odour emission using weeks as a factor (Across all treatment groups) showed that there was a marginal but significant difference between week 1 and week 2 ($R=0.225$; $P<0.05$) and week 1 and week 3 ($R=0.463$; $P<0.05$) were significantly different. However, there was no significant difference between week 2 and week 3 ($R=0.093$; $P>0.05$). The test for treatment groups (across all week groups) showed that there were no significant difference across all treatment groups (global $R=0.026$; $P>0.05$). The test for differences on odour emission using feed type as a factor (Across all week groups) indicated that there was a significant differences between starter and grower ($R=0.282$; $P<0.05$). However, there were no significant differences between starter and finisher ($R=0.107$; $P>0.05$), and grower and finisher ($R=0.089$; $P>0.05$).

A two way simper analysis using week as a factor (Across all feed type groups) indicated that decanal, nonanal, 4-methyl phenol, acetic acid amide, 1-Octanol, acetamide, N-methyl (Methylacetamide), phenylethyl alcohol, 2-methoxy phenol (Mequinol), 3-methyl-phenol, benzaldehyde, octanal, hexanoic acid, heptanoic acid , heptanal, isocrotonic acid, pentanoic acid, hexanal and acetoin characterized the emissions across the different litters as affected by feed type and accounts for 90% of average similarity in week 1. In week 2 nonanal, decanal, 1-butanol, octanal, phenylethyl alcohol, 4-methyl phenol, heptanal, verbenone, dimethyl disulphide, isocrotonic acid, heptanoic acid, Acetamide, N-methyl (Methylacetamide), 2-methoxy phenol (Mequinol) and Acetic acid amide accounted for 91% of average similarity. Moreover, nonanal, octanal, 1-butanol, decanal, heptanal, cetic acid amide, 2-methoxy phenol (Mequinol), hexanal, pentanoic acid and octanoic acid accounted for 90% average similarity in week 3.

Table 3.3a. Effect of EM treatments on volatile organic compounds (Alcohol and Aldehyde) concentrations from beef manure in relation to feed diet over a three week period.

COMPOUND CIASS		ALCOHOLS					ALDEHYDE					
		1-butanol	1-Nonanol	1-hexanol	1-octen-3-ol	1-Octanol	Hexanal	Heptanal	Octanal	Nonanal	Decanal	(E)-2-Nonenal
KIR		1141	1292	1352	1444	1582	1090	1180	1278	1388	1531	1568
CAS		71-36-3	143-08-8	111-27-3	3391-86-4	111-87-5	66-25-1	111-71-7	124-13-0	124-19-6	112-31-2	18829-56-6
W1	ST0	0.72	-	-	0.52	0.29	1.17	1.17	1.41	4.46	21.76	0.03
	ST1	0.05	-	31.16	0.05	0.01	1.87	1.25	0.81	1.36	0.11	0.04
	ST2	-	-	0.15	0.13	-	1.23	-	1.10	0.45	0.06	-
	ST3	3.06	-	-	0.48	-	4.00	2.76	3.22	5.97	2.54	0.11
W2	ST0	0.77	-	-	0.90	0.52	0.67	0.95	3.74	9.20	7.38	0.13
	ST1	13.49	-	0.22	0.31	-	0.86	1.31	2.86	7.76	0.70	-
	ST2	0.55	-	2.02	0.44	0.07	1.00	0.78	5.11	4.54	1.14	0.67
	ST3	0.56	-	-	0.60	-	0.68	2.15	2.29	3.43	2.08	0.05
W3	STO	19.44	-	-	-	0.32	2.91	3.08	5.00	11.92	1.92	-
	ST1	6.58	-	-	-	0.56	1.66	3.12	6.79	25.74	7.41	1.31
	ST2	31.31	-	-	-	-	5.57	4.58	7.62	12.64	2.30	1.03
	ST3	21.67	-	-	-	-	3.42	1.16	4.63	12.35	3.81	-
W1	GTO	0.58	1.29	1.20	0.54	-	6.66	7.32	9.97	20.27	3.27	-
	GT1	4.70	-	1.06	0.33	-	4.25	8.59	4.14	15.15	4.13	-
	GT2	0.68	5.01	-	-	-	-	0.36	2.04	11.25	6.52	-
	GT3	4.16	2.90	-	0.02	-	0.41	-	-	7.83	2.61	0.18
W2	GT0	22.38	-	-	-	-	-	1.23	6.80	13.91	4.09	-
	GT1	14.79	-	-	-	-	-	1.60	7.31	16.12	7.16	-
	GT2	0.68	5.01	-	-	-	-	0.36	2.04	11.25	6.52	-
	GT3	4.16	2.90	-	0.02	-	0.41	-	-	7.83	2.61	0.18
W3	GT0	1.36	-	-	-	-	1.80	1.84	7.30	40.65	10.38	0.24
	GT1	41.65	-	-	-	-	-	6.63	15.73	18.34	2.37	0.79
	GT2	17.97	-	-	-	-	1.96	7.04	47.10	13.78	1.87	-
	GT3	37.71	-	-	-	-	-	0.88	4.70	10.45	3.36	0.01
W1	FTO	-	-	-	0.44	-	2.33	1.69	0.08	3.37	0.17	-
	FT1	0.13	0.04	-	-	-	-	20.84	34.67	4.25	0.98	-
	FT2	-	0.19	-	0.23	-	0.09	-	-	0.99	0.26	-
	FT3	4.42	-	-	-	-	-	0.14	0.09	0.38	0.12	-
W2	FT0	7.78	-	-	3.19	-	-	3.58	1.71	41.06	5.02	0.44
	FT1	56.34	-	-	-	0.22	-	-	3.67	11.66	3.34	-
	FT2	12.99	-	-	-	1.08	13.13	2.28	6.29	17.20	3.92	-
	FT3	0.35	-	-	-	-	-	1.43	2.10	2.49	-	-
W3	FT0	13.92	-	-	0.14	-	-	5.11	36.25	9.88	3.39	-
	FT1	22.60	-	-	-	1.41	-	2.45	5.91	21.78	8.15	0.79
	FT2	13.92	-	-	-	-	-	2.96	10.69	49.59	16.92	0.88
	FT3	21.36	-	-	-	-	1.41	1.83	5.38	13.12	4.12	-

ST0= Starter treatment control (0% EM); ST1= Starter treatment 1 (10% EM); ST2= Starter treatment 2 (20% EM); ST3= Starter treatment 3 (30% EM); GT0= Grower treatment control (0% EM); GT1= Grower treatment 1 (10% EM); GT2= Grower treatment 2 (20% EM); GT3= Grower treatment 3 (30% EM); FT0= Finisher treatment control (0% EM); FT1= Finisher treatment 1 (10% EM); FT2= Finisher treatment 2 (20% EM); FT3=Finisher treatment 3 (30% EM). W1= sampling on the first week; W2= sampling after 2 weeks; W3= sampling after 3 weeks. Compounds were identified by common names and CAS (Chemical Abstract Service) registry number, and listed according to estimated Kovats retention index (KRI) within each compound class. Values are mean percentages of the total peak area.

Table 3.3b. Effect of EM treatments on volatile organic compounds (Volatile fatty acids) concentrations from beef manure in relation to feed diet over a three week period.

COMPOUND CLASS		VOLATILE FATTY ACIDS														
		Acetic A	Propanoic A	Isobutyric A	Butanoic A	2-Methylhexanoic A	2-Methylbutanoic A	Pentanoic A	Isocrotonic A	4-methyl-pentanoic A	Hexanoic A	Heptanoic A	Octanoic A	Nonanoic A	Dodecanoic A	Benzoic A
KRI		1458	1537	1604	1645	1670	1672	1758	1773	1844	1885	1952	2094	2197	2454	2471
CAS		64-19-7	79-09-4	79-31-2	107-92-6	4536-23-6	116-53-0	109-52-4	503-64-0	646-07-1	142-62-1	111-14-8	124-07-2	112-05-0	143-07-7	65-85-0
W1	ST0	14.32	3.09	1.38	23.85	3.21	5.66	2.27	-	2.11	0.98	0.11	0.49	0.34	0.05	-
	ST1	14.39	2.10	1.02	12.60	-	2.10	0.39	23.85	0.10	0.06	0.01	0.02	0.02	0.01	-
	ST2	1.34	3.56	1.29	35.52	0.43	3.81	3.40	0.19	2.32	1.93	0.06	0.31	0.26	0.01	-
	ST3	4.86	3.64	0.78	32.54	4.24	3.27	3.49	0.29	2.33	2.90	0.66	0.86	1.30	0.02	0.23
W2	ST0	13.53	-	1.56	0.26	-	-	-	0.09	9.49	0.27	-	-	-	-	-
	ST1	24.83	3.54	2.10	18.56	-	4.44	1.84	0.02	0.14	0.20	-	-	-	0.06	-
	ST2	16.65	2.57	3.21	10.98	-	7.59	1.68	-	1.33	-	2.15	0.51	0.72	-	-
	ST3	5.12	1.62	0.51	10.11	-	7.58	0.43	-	1.02	0.35	0.12	-	-	-	-
W3	STO	9.48	-	0.56	0.99	3.25	-	1.79	-	0.13	0.09	0.02	1.37	-	-	-
	ST1	0.31	-	0.49	3.95	32.96	-	1.03	-	0.87	0.84	0.34	0.60	0.52	-	-
	ST2	9.97	2.45	1.20	-	2.69	1.56	4.08	-	0.89	1.19	-	0.15	-	-	-
	ST3	40.66	-	0.57	-	0.79	0.56	0.59	-	-	0.65	-	-	-	-	-
W1	GTO	2.67	-	0.65	0.63	-	-	0.40	-	-	-	-	-	-	-	-
	GT1	1.23	-	12.73	10.56	8.81	-	3.18	-	2.40	1.69	-	1.38	1.11	-	-
	GT2	1.93	1.82	0.57	2.75	0.82	1.46	17.75	-	2.80	1.22	0.70	-	2.04	-	-
	GT3	5.88	0.86	0.88	2.41	-	1.21	0.76	-	0.45	0.67	0.12	-	0.87	-	-
W2	GT0	6.47	-	-	2.71	2.39	1.76	0.97	-	0.20	4.38	-	2.40	-	-	0.64
	GT1	4.48	-	-	1.43	0.02	-	0.58	-	-	5.20	2.26	3.33	4.94	-	-
	GT2	1.93	1.82	0.57	2.75	0.82	1.46	17.75	-	2.80	1.22	0.70	-	2.04	-	-
	GT3	5.88	0.86	0.88	2.41	-	1.21	0.76	-	0.45	0.67	0.12	-	0.87	-	-
W3	GT0	0.76	-	0.22	-	-	1.47	-	-	0.94	2.01	0.53	1.93	6.11	1.63	-
	GT1	1.99	-	0.17	-	2.34	0.00	-	-	0.68	0.91	-	-	-	-	-
	GT2	3.19	-	0.47	-	0.93	-	0.86	-	-	1.82	-	-	0.54	-	-
	GT3	1.81	-	-	-	-	0.01	0.48	-	-	0.01	0.01	-	-	0.27	-
W1	FTO	6.04	4.18	1.20	56.79	1.95	9.22	4.23	0.09	0.56	0.37	0.03	0.03	0.05	0.01	0.01
	FT1	0.18	0.12	0.10	0.73	0.23	-	0.82	-	1.04	0.58	-	0.44	29.98	-	-
	FT2	4.69	4.07	1.31	31.95	1.93	1.14	2.97	0.06	1.26	0.32	0.02	0.05	0.07	0.01	-
	FT3	15.28	5.86	3.03	29.15	0.57	1.27	1.07	-	0.32	0.12	-	-	-	-	-
W2	FT0	7.56	0.75	0.39	12.91	1.45	1.68	0.23	-	0.19	-	0.01	-	-	-	-
	FT1	2.94	-	-	-	1.30	-	-	-	-	4.54	-	-	-	0.20	-
	FT2	6.13	-	-	2.49	7.23	-	10.97	0.88	0.42	1.36	-	-	-	-	-
	FT3	10.51	2.56	0.46	2.75	1.29	-	0.97	-	-	-	-	-	-	-	-
W3	FT0	2.04	-	0.68	20.69	2.18	-	1.25	-	0.45	0.19	0.14	0.06	-	0.06	-
	FT1	5.40	-	-	-	-	-	29.12	-	-	0.81	0.04	0.11	0.13	0.13	-
	FT2	0.28	-	-	-	-	-	0.26	-	0.43	0.45	-	-	-	-	-
	FT3	0.58	-	-	-	32.46	-	0.34	-	0.52	1.51	0.93	1.28	1.44	0.20	-

ST0= Starter treatment control (0% EM); ST1= Starter treatment 1 (10% EM); ST2= Starter treatment 2 (20% EM); ST3= Starter treatment 3 (30% EM); GT0= Grower treatment control (0% EM); GT1= Grower treatment 1 (10% EM); GT2= Grower treatment 2 (20% EM); GT3= Grower treatment 3 (30% EM); FT0= Finisher treatment control (0% EM); FT1= Finisher treatment 1 (10% EM); FT2= Finisher treatment 2 (20% EM); FT3= Finisher treatment 3 (30% EM); A= Acid; W1= sampling on the first week; W2= sampling after 2 weeks; W3= sampling after 3 weeks. Compounds were identified by common names and CAS (Chemical Abstract Service) registry number, and listed according to estimated Kovats retention index (KRI) within each compound class. Values are mean percentages of the total peak area.

Table 3.3c. Effect of EM treatments on volatile organic compounds (Ketones and phenolics) concentrations from beef manure in relation to feed diet over a three week period.

COMPOUND CLASS		KETONES					PHENOLICS								
		2-Heptanone	3-Octanone	Acetoin	2-Octanone	2-Nonanone	Mequinol	2-Hydroxy-4-methylbenzaldehyde	Phenylethyl alcohol	Phenol	4-methyl phenol	3-methyl-phenol	4-ethyl phenol	3-Ethyl phenol
	KRI	1198	1249	1277	1284	1395	1865	1903	1937	2043	2076	2080	2079	2167
	CAS	110-43-0	106-68-3	513-86-0	111-13-7	821-55-6	90-05-1	698-27-1	60-12-8	108-95-2	106-44-5	108-39-4	123-07-9	620-17-7
	ST0	0.11	-	0.35	-	-	-	-	0.03	1.08	0.58	7.15	-	-
	ST1	-	-	3.68	-	-	-	-	0.01	0.96	1.12	0.31	-	-
	ST2	-	-	-	-	0.65	-	-	0.27	34.94	5.01	0.02	-	-
W1	ST3	-	-	0.47	-	-	0.03	-	0.03	3.32	7.80	0.51	-	-
	ST0	-	-	-	-	-	-	-	0.18	3.15	-	11.40	-	0.22
	ST1	-	-	1.03	-	-	-	-	0.24	2.84	1.18	5.07	0.13	-
	ST2	-	-	0.35	-	-	-	-	0.24	4.12	7.90	3.95	0.26	-
W2	ST3	-	-	-	-	-	-	-	0.18	3.33	-	16.63	0.10	0.29
	STO	-	-	-	-	-	-	-	0.02	0.58	0.23	2.27	0.02	-
	ST1	-	-	0.98	-	-	-	-	0.11	0.86	-	1.40	-	-
	ST2	-	-	-	-	-	-	-	0.17	1.78	-	4.63	-	-
W3	ST3	-	-	-	-	-	-	-	-	0.66	0.02	1.00	-	-
	GTO	6.03	3.74	-	0.70	-	-	-	-	0.68	0.79	0.16	-	-
	GT1	1.26	2.50	-	0.33	-	-	-	0.22	1.08	0.88	0.08	-	-
	GT2	-	-	-	5.03	0.51	-	-	-	0.72	0.41	0.24	-	-
W1	GT3	7.47	2.06	-	-	-	0.06	-	-	17.36	0.80	0.24	-	-
	GT0	-	-	-	-	-	-	-	-	0.35	-	1.35	-	-
	GT1	-	-	-	-	-	-	-	-	0.36	-	0.23	-	-
	GT2	-	-	-	5.03	0.51	-	-	-	0.72	0.41	0.24	-	-
W2	GT3	7.47	2.06	-	-	-	0.06	-	-	17.36	0.80	0.24	-	0.05
	GT0	-	-	-	-	-	-	-	-	0.30	8.53	0.26	-	-
	GT1	-	-	-	-	-	-	-	-	0.40	-	-	-	-
	GT2	-	-	-	-	-	-	-	-	-	-	0.24	-	-
W3	GT3	-	-	-	-	-	-	-	-	0.02	-	-	-	-
	FTO	-	0.01	0.24	0.21	0.04	-	-	0.34	1.68	3.57	0.02	-	-
	FT1	-	0.28	-	-	-	0.02	-	0.45	1.23	1.28	0.34	-	-
	FT2	0.59	-	4.06	-	-	0.01	-	0.20	1.09	0.79	4.05	-	0.04
W1	FT3	-	-	3.45	-	-	-	-	0.01	33.87	0.69	-	-	-
	FT0	-	-	1.05	-	-	-	-	0.48	2.20	-	4.17	-	-
	FT1	-	-	-	-	-	-	-	-	-	-	0.16	0.34	-
	FT2	-	-	-	-	-	-	-	-	0.42	1.22	1.73	0.08	-
W2	FT3	-	-	0.15	-	-	0.01	-	0.20	1.40	5.26	0.26	0.26	0.02
	FT0	-	-	-	-	-	-	-	-	0.62	-	0.01	-	0.04
	FT1	-	-	-	-	-	-	-	-	0.02	-	-	-	-
W3	FT2	-	-	-	-	-	-	-	-	-	0.29	0.45	-	-
	FT3	-	-	-	-	-	-	-	-	-	-	0.01	-	-

ST0= Starter treatment control (0% EM); ST1= Starter treatment 1 (10% EM); ST2= Starter treatment 2 (20% EM); ST3= Starter treatment 3 (30% EM); GT0= Grower treatment 0 (control); GT1= Grower treatment 1 (10% EM); GT2= Grower treatment 2 (20% EM); GT3= Grower treatment 3 (30% EM); FT0= Finisher treatment 0 (control); FT1= Finisher treatment 1 (10% EM); FT2= Finisher treatment 2 (20% EM); FT3= Finisher treatment 3 (30% EM); W1= sampling on the first week; W2= sampling after 2 weeks; W3= sampling after 3 weeks. Compounds were identified by common names and CAS (Chemical Abstract Service) registry number, and listed according to estimated Kovats retention index (KRI) within each compound class. Values are mean percentages of the total peak area.

Table 3.3d. Effect of EM treatments on volatile organic compounds (Terpenoids and amide) concentrations from beef manure in relation to feed diet over a three week period.

COMPOUND CLASS		TERPENOIDS					AMIDE		
		Eucalyptol	β-Gurjunene	4-ketoisophorone	Verbenone	Isomethyl ionone	Acetamide, N-methyl (Methylacetamide)	Acetic acid amide	butanamide
	KRI	1215	1652	1704	1725	1872	1629	1750	1887
	CAS	470-82-6	17334-55-3	1125-21-9	1196-01-6	127-51-5	79-16-3	60-35-5	541-35-5
W1	ST0	-	-	0.18	-	-	-	-	0.02
	ST1	-	-	-	-	-	-	-	-
	ST2	-	-	-	0.03	-	-	-	0.09
	ST3	-	-	0.03	-	-	-	-	0.06
W2	ST0	-	-	2.07	-	-	-	-	0.08
	ST1	-	-	0.04	-	-	0.53	0.10	0.14
	ST2	-	-	0.72	-	-	1.47	0.58	-
	ST3	-	-	9.89	-	-	0.32	-	0.21
W3	STO	-	-	-	-	-	0.13	-	0.02
	ST1	-	-	-	-	-	-	-	-
	ST2	-	-	0.09	-	-	-	-	-
	ST3	-	-	-	-	-	-	-	-
W1	GTO	-	-	3.10	0.56	-	-	-	-
	GT1	-	-	1.22	0.23	-	-	-	-
	GT2	-	-	0.13	-	1.74	-	-	-
	GT3	-	-	0.36	36.66	-	1.40	-	-
W2	GT0	-	-	4.14	0.46	5.20	-	-	-
	GT1	-	-	0.85	0.32	-	-	-	-
	GT2	-	-	0.13	-	1.74	-	-	-
	GT3	-	-	0.36	36.66	-	1.40	-	-
W3	GT0	-	-	0.54	-	-	0.10	-	-
	GT1	0.57	-	0.79	-	0.14	-	-	-
	GT2	1.60	-	-	-	-	-	-	-
	GT3	2.05	-	1.33	0.34	-	-	-	-
W1	FTO	-	-	-	-	0.37	-	-	0.06
	FT1	-	-	-	-	-	0.15	-	-
	FT2	-	-	-	-	-	-	-	0.06
	FT3	-	-	-	-	-	-	-	-
W2	FT0	-	-	0.01	-	-	-	-	-
	FT1	0.45	-	-	-	-	-	-	-
	FT2	3.49	-	-	-	-	-	-	-
	FT3	1.20	-	-	0.17	-	0.79	-	-
W3	FT0	-	-	-	-	0.33	-	-	0.14
	FT1	-	-	-	-	0.04	-	-	-
	FT2	1.78	-	-	-	-	-	-	-
	FT3	0.78	-	-	-	0.47	-	-	-

ST0= Starter treatment control (0% EM); ST1= Starter treatment 1 (10% EM); ST2= Starter treatment 2 (20% EM); ST3= Starter treatment 3 (30% EM); GT0= Grower treatment control (0% EM); GT1= Grower treatment 1 (10% EM); GT2= Grower treatment 2 (20% EM); GT3= Grower treatment 3 (30% EM); FT0= Finisher treatment control (0% EM); FT1= Finisher treatment 1 (10% EM); FT2= Finisher treatment 2 (20% EM); FT3= Finisher treatment 3 (30% EM); W1= sampling on the first week; W2= sampling after 2 weeks; W3= sampling after 3 weeks. Compounds were identified by common names and CAS (Chemical Abstract Service) registry number, and listed according to estimated Kovats retention index (KRI) within each compound class. Values are mean percentages of the total peak area.

Table 3.3e. Effect of EM treatments on volatile organic compounds (Sulphur and nitrogen compound) concentrations from beef manure in relation to feed diet over a three week period.

COMPOUND CLASS		SULPHUR COMPOUND			NITROGEN COMPOUND			
		Dimethyl disulphide	Dimethyl trisulphide	Dimethyl sulfone	Pyridine	Indole	2-Pyrrolidinone	2-Piperidinone
	KRI	1074	1376	1941	1387	2421	2070	2127
	CAS	624-92-0	3658-80-8	67-71-0	1462-84-6	120-72-9	616-45-5	675-20-7
W1	ST0	-	-	0.95	-	-	-	-
	ST1	-	-	0.04	-	-	-	-
	ST2	-	-	0.09	-	-	-	-
	ST3	-	-	0.65	-	-	-	-
W2	ST0	-	-	0.76	-	-	-	-
	ST1	-	-	1.86	-	-	0.04	-
	ST2	0.81	-	2.47	4.67	-	-	-
	ST3	-	-	29.05	-	-	0.16	0.04
W3	STO	-	-	3.51	1.69	-	28.36	-
	ST1	-	-	0.81	-	-	-	-
	ST2	-	-	0.72	-	-	0.05	-
	ST3	-	-	-	0.73	-	4.52	-
W1	GTO	11.71	8.23	3.85	-	-	-	-
	GT1	1.79	1.31	1.48	-	-	-	-
	GT2	-	0.09	-	-	-	-	-
	GT3	-	-	0.71	-	-	-	-
W2	GT0	2.19	1.68	-	-	-	1.35	-
	GT1	6.92	0.04	-	0.90	-	-	-
	GT2	-	0.09	-	-	-	-	-
	GT3	-	-	0.71	-	-	-	-
W3	GT0	-	-	-	1.48	-	2.91	-
	GT1	-	-	1.16	0.58	-	0.54	-
	GT2	0.02	-	0.42	-	-	-	-
	GT3	-	-	1.49	0.21	-	2.30	-
W1	FTO	-	0.10	0.02	-	0.01	-	-
	FT1	-	-	0.02	-	-	-	-
	FT2	-	-	0.03	-	-	-	-
	FT3	-	-	-	-	-	-	-
W2	FT0	-	0.04	-	-	-	0.36	-
	FT1	10.74	0.04	-	-	-	-	-
	FT2	1.17	0.42	-	-	-	0.29	-
	FT3	33.30	-	-	-	-	-	-
W3	FT0	-	0.32	0.24	0.01	-	-	-
	FT1	-	-	-	0.19	-	0.85	-
	FT2	-	-	0.57	0.06	-	0.36	-
	FT3	10.44	-	0.01	0.01	-	-	-

ST0= Starter treatment control (0% EM); ST1= Starter treatment 1 (10% EM); ST2= Starter treatment 2 (20% EM); ST3= Starter treatment 3 (30% EM); GT0= Grower treatment 0 control (0% EM); GT1= Grower treatment 1 (10% EM); GT2= Grower treatment 2 (20% EM); GT3= Grower treatment 3 (30% EM); FT0= Finisher treatment 0 control (0% EM); FT1= Finisher treatment 1 (10% EM); FT2= Finisher treatment 2 (20% EM); FT3= Finisher treatment 3 (30% EM); W1= sampling on the first week; W2= sampling after 2 weeks; W3= sampling after 3 week. Compounds were identified by common names and CAS (Chemical Abstract Service) registry number, and listed according to estimated Kovats retention index (KRI) within each compound class. Values are mean percentages of the total peak area

3.4 Discussion

The aim of this study was to investigate the effect of feed type and effective microorganisms on feedlot cattle performance and litter gas emissions (particularly VOCs). This is because most researchers have been focusing on; (a) feed formulation to optimize animal performance an ignoring emissions; (b) mitigating on fewer emissions when attempted (Ammonia (NH3), methane (CH4), and Hydrogen sulphide (H2S) (Li et al., 2012, Gerber et al., 2013) from livestock while giving a blind eye on other potential emissions such as VOCs and (c) most mitigation studies (Conti et al., 2019) pay less attention to EM hence the need for EM to be exploited. It is very rare to find beef producers who are ready to change their animals' diet to mitigate VOCs emissions because feed is one the largest input cost for production (Woodbury et al., 2014). However, there is a need to consider it as demonstrated by the results observed in this study where the highest number of gaseous emission were found in week 2 of starter diet which was richer in solubles than fibre. Sometimes cost is an issue and farmers will turn to look at lowest cost rations regardless of its impacts on gaseous emissions. This study monitored emissions from litter changing diet as well as emissions from EM treated diets as an attempt to mitigate emissions to undetectable amounts.

The type of diet provided to the beef cattle had significant impact on the animal performance since the weight gain and feed intake per kg of cattle body mass and feed conversion ratio increased as animal changed diet from starter, grower to finisher. This is due to the feed composition of the diet, which consisted of available carbohydrates (Starch, hemicellulose and other soluble) and least

available carbohydrates (cellulose and lignin) for energy and development but when exposed to rumen microbes for fermentation and degradation of plant fibres (Myer et al., 2017). High fibre diets fed to ruminants have the potential to improve microbial protein synthesis required for growth and development which is the primary goal for most (if not all) beef producers (Nathani et al., 2015). Therefore, the relatively higher intake and weight gain per kg of body mass observed in grower and finisher than in starter was due to the high fibre content which is a conducive environment for rumen fibrolytic microbes because it is properly buffered for optimal activities. However, the diet composition is also said to have had an impact on gaseous and odour emission.

Beef manure is considered fresh if it is less than 24 hours old (<22 h) and regarded as aged or old when older than 3 weeks where by it losses moisture, pH changes, and there are less substrates (CP, starch, non-starch carbohydrates) for fermentation and fermentation microbes can no longer survive (Miller and Varel, 2001). However, this study has proven otherwise by trapping numerous gases after three weeks hence the presence of activities even after three weeks. The list of VOCs emitted by cattle manure in this study agrees with literature as reported to be emitted by livestock premises (Cai et al., 2006, Hales et al., 2012, Bastami, 2016). Amongst them, acetic was highlighted in swine and beef manure as highly corrosive to the skin and eyes of people (Agarwal et al., 2018). Acetic acid can also be damaging to the internal organs if ingested or in the case of vapour inhalation by human (Zhang et al., 2020). Hence, it is considered a toxic gas from cattle manure that needs to be monitored and mitigated. In another study, Zhang et al. (2020) observed

that the main odour VOCs in the manure composting process were phenol, dimethyl sulphide and dimethyl trisulphide and these were also captured in this study. Romagnoli et al. (2014) reported that short-term exposure to phenol in the air can cause respiratory irritation, headaches, and burning eyes. If there is only a short term exposure, then it is clear that this gas may have an effect on the day to day workers on the farm due to the high emissions observed in week 1. Animal studies have reported that offspring's of animals exposed to phenol orally, may result in reduced foetal body weights, growth retardation, and abnormal development (Romagnoli et al., 2014). Phenol was not administered orally to the animals but the big question is, what about inhalation? Le et al. (2005) reported that sulphurous compounds are the most offensive compounds, the odorous nature of this compounds progresses from the putrid smell of dimethyl disulphide and dimethyl trisulphide to the rotten eggs smell of hydrogen sulphide. Apart from hydrogen sulphide, dimethyl disulphide, dimethyl sulfone and dimethyl trisulphide were also very important odourous compounds to be considered for mitigation. However, EM at treatment level 3 (T3) reduced the sulphides into undetectable quantities but for dimethyl sulfone.

Acetic acid, phenol, dimethyl sulphide and dimethyl trisulphide were mitigated by the use of EM at all treatment levels to undetectable amounts in week 3 compared to the control. However, in week 2 dimethyl disulphide was observed to increase to high concentration in treatment FT3 (EM 30%) compared to the control. This was probably due to deamination of microbial protein

(particularly, methanethiol, L-Cysteine, and L-Methionine) since the finisher feed protein content was the lowest (Le Phung et al., 2005).

The manure from beef cattle fed with grower had more sulphur compounds and VFAs compared to starter manure. This could be explained by the fact that, during the grower feeding phase the animal was fed with a ration that contained higher amounts of crude protein and fibre (NDF, ADF, HEM) content compared to starter and finisher which implies that only a portion of the diet will be absorbed by the animal and the rest excreted via urine and faeces which may be utilized as substrates for fermentation in the manure (Le et al., 2005). Hence resulting in high odour (Phenol, dimethyl disulphide, dimethyl trisulphide and dimethyl sulfone) emission in grower manure compared to starter. Surprisingly, the number of odorous compounds emitted from manure of beef fed with finisher diet increased while the protein content was lower compared to grower manure. This may be due to decreased energy and protein concentrates when compared to fibre hence a lot of the fibre passes through partially digested or undigested. This undigested fibre serves as a substrate for fermentation which lead to relatives higher VOCs (especially acetic acid) when compared to the grower (Sutton et al., 2006).

The use of EM at different treatment levels (10% EM, 20% EM, and 30% EM) to mitigate gaseous emissions from beef manure showed that there was a significant difference between the control and EM treated samples but no significant difference within treatment levels. This means that EM

do not depend solely on the initial concentration (dose) to reduce gaseous emissions from beef manure but seems to depends on time since there was no significant difference in gases emitted between week 1 and week 3 across all EM treated feed types. This may be due to increased effective microorganism population by multiplication with time which also implies that more toxic microbes or bad microorganisms were also being eliminated in the manure. Bastami (2016) reported that cattle manure amendment by various concentrations of effective microorganisms (EM10%, EM20% and EM30%) resulted in only a small reduction in methane emissions for a short period (three days). This implies that EM requires time for the microbes to compete with microbes within the manure system. The significant drop of certain compounds in week 3 (VFAs, sulphur compounds, nitrogen compounds and phenolics) implies that EM might have reduced or eliminated the particular microbes that thrives on specific substrates or by-products to produce them. Some aldehydes such as hexanal has been observed being used in the flavour industry to produce fruity flavors. Its scent resembles freshly cut grass (Swaine Jr, 1995, Xiao et al., 2016). Nonanal is a clear brown liquid characterized by a rose-orange odour. It is found in at least 20 essential oils, including rose and citrus oils and several species of pine oil. It is mainly used in perfumes (Bauer et al., 2008). Decanal is a saturated fatty aldehyde formally arising from reduction of the carboxy group of capric acid (decanoic acid) and has been associated with antifungal activities by Arctander (2019). Effective microorganisms also overcome the odour producing microbes in animal manure (such as sulphide producing bacteria) by replacing them with the

beneficial microbes that are contained in EM (Davis et al., 2013). Further research needs to be conducted to identify the type and amount of microbes that thrives in cattle manure and what EM eliminates as well as their interaction and mechanism of toxicity to microbes and odour reduction.

3.5 Conclusion

From this study, it can be concluded that diet type plays a major role in odour emission in beef cattle manure. Gaseous emissions varied with type of diets from starter, grower to finisher. The highest number of gases were observed in the litter from animals fed with grower diet. Effective microorganism treatment of cattle manure generally reduced gaseous emissions but dose quantity preferred was 10% as higher doses effect was minimal. Livestock odour does not come from an individual compound but from a complex mix of various compounds as demonstrated by the multi-odorous compounds reported in this study. The main odour producing compounds were VFAs, sulphurous compounds, and phenols. Amongst these compounds, acetic acid and phenol was identified as the dominant toxic compound to human kind. Dimethyl sulphide and dimethyl trisulphide were detected as the main dominant odour causing compounds in cattle with huge toxicity potential. The majority of these compounds were successfully reduced to indictable levels or mitigated by the use of EM. However, dimethyl trisulphide was poorly mitigated. Therefore, more strategies on how to reduce this gas needs to be explored.

CHAPTER FOUR: Effect of feed type and effective microorganisms on pig performance and litter gaseous emissions, respectively.

Abstract

Pig litter is one of the major sources of odour emission in livestock farming. However, most studies have focused on ammonia and a few on hydrogen sulphide as odour causing compounds. Odour can be a mixture of various volatile organic compounds coming from manure but has not been exploited. Therefore, identification, source and mitigating strategies of other odour causing compounds from pig's litter remains unclear. Therefore, the objective of this study was to determine the effect of feed type and effective microorganisms on pig performance and litter gaseous emissions, respectively. A total of 50 large white landrace x duroc piglets weaned at 31 days of age with average body mass of 7kg housed in five different pens were used in this study. Pigs were fed starter, grower and finisher diets while intake, and weight gain and feed conversion ratio were the performance parameters measured. Faeces were collected randomly at 20 faecal deposits, mixed and divided into four groups with replicates before treating with EM at different concentrations; 0% EM (Control); 10% EM; 20% EM; and 30% EM. The results showed that diet clearly improved ($P<0.05$) the performance of pig since the weight gain, feed intake and feed conversion ratio increased as the animal moves from one diet to another. Diet had an effect ($P<0.05$) on gaseous emissions from pig manure since the total gases emitted for grower litter (40) was higher ($P<0.05$) than in the finisher litter (38) but less than in starter (41). The gaseous compounds detected in the manure included a high amount of volatile fatty acids (VFAs), Sulphur, Nitrogen containing and phenolic compounds. P-Cresol (4-methyl phenol) and phenol were two hazardous air pollutants identified while indole and skatole were identified to be responsible for boar taint in pigs. The use of EM at different concentrations indicated that there were no

differences in treatment groups (across feed type groups) but differed ($P<0.05$) from the control. However, there was a significant difference in odour emitted between week 1 and week 2 ($R=0.614$; $P <0.05$); week 1 and week 3 ($R=0.536$; $P<0.05$); and week 2 and week 3 ($R=0.25$; $P<0.01$). This implies that there was a reduction of gases from manure using EM but it was time dependent as the microbes to adapt, grow and multiply to compete with the microbes in swine manure. It was concluded that 30% EM dose treatment was recommended for rapid gas mitigation early in week 2 but most compounds were undetectable in week 3. However, Dimethyl disulphide was not mitigated even at treatment 30% EM hence more alternative methods on how to mitigate this compound need to be explored.

Keywords: Effective microorganisms, odour, volatile organic compounds, fibre, litter, boar taint, phenol, indole, skatole.

4.1 Introduction

Intensive animal production, especially swine production, generate minerals, odour, volatile organic compounds, ammonia and dust which usually exceed levels tolerated by the human population (Akdeniz et al., 2012). This has resulted in more complaints reported in many countries which is about 20% yearly. However, approximately 40% of the complaints are from pig farms and 35% from poultry farms (Mielcarek and Rzeznik, 2015). There are a number of reasons for the increased number of complaints: (a) increased sizes of the farms and the number of livestock, especially in areas where both animals and humans are concentrated (b) increased in residential development near traditionally agricultural industrial areas. (c) unpleasant odours causing a variety of emotional and undesirable reactions in people, ranging from annoyance to documented health effects, leading to a reduced quality of life and (d) increased sensitivity and demand of the general public for a clean and pleasant environment (Rappert and ller, 2005, Conti et al., 2019).

These reasons have forced the pig industries to start investing in strategies to mitigate odour emissions, as well as toxic air pollutants (Conti et al., 2019). Emissions of odour from swine production originate from: (a) diet; (b) animal bodies; and (c) urine and faeces or the mixture of both. Diet contributes more to the variation of odour, because its composition is directly related to odour production (Mpendulo et al., 2018). Livestock manure consists of undigested organic residues, including proteins, carbohydrates, and fats. These components can be degraded anaerobically in the manure, which results in the emissions of ammonia, hydrogen sulphide and volatile organic compounds (i.e. N compounds, volatile fatty acids (VFA), sulphur compounds, various alcohols, and aromatic (indole, skatole, and cresol compounds) (Varel, 2002). These compounds are considered as the main volatile components from livestock production facilities with any appreciable odour (Varel, 2002). Ammonia and hydrogen sulphide are not well correlated with odour intensity. However, Zahn (1997) managed to report a good correlation between air concentrations of VOCs and odour offensiveness when 34 swine production facilities were studied. Therefore, controlling the formation and mitigating the emission of VOCs should be well explored since they have a direct influence on odours released from animal production facilities.

Feed additives such as effective microorganisms (EM) are one of the biochemical and chemical agents that can be used to reduce odour from animal production. Amon et al. (2006b) reported that the application of EM in cattle slurries relatively reduced the emissions of NH_3 and N_2O, but slightly increased CH_4. This showed the potential of EM as an agent to reduce NH_3 and greenhouse gas emissions from stored slurry even though volatile organic compounds were not considered. Mafiri (2014) conducted an experiment determining the effect of supplementing diets with effective microorganisms on performance of male Ross 308 broiler chickens. The results indicated that EM supplementation improved crude protein retention and crude protein content of chicken

meat. Supplementing chicken diet with effective microorganisms also reduced mortality of the chickens from 5 to 0 %. This demonstrated that EM can improve animal performance. However, it would have been great to explore the potential of EM in mitigating odour and other gaseous emission from the litter. Bastami (2016) obtained cattle manure or slurry from a commercial farm in two different seasons (winter and summer) and treated it with a mixture of actiferm EM and glucose at different levels and reported a decrease in greenhouse gas (CH_4, CO_2, and N_2O) emissions during 120 days of winter compared to summer storage. However, the use of EM to reduce volatile organic compounds emitted from swine manure has not been well explored. Hence the aim of this study was to evaluate the effect of different diets on swine performance and litter gaseous emissions after treatment with EM.

4.2 Materials and Methods

4.2.1 Study site

The Feeding trial was conducted in Bayensfield piggery, Pietermaritzburg, KwaZulu-Natal, South Africa. Bayensfield piggery is located within the latitude of -29.6168 and longitude of 30.3928, with an altitude of 764.7432m. The daily temperatures average 29°C, with variation ranging from 28.2 to 43°C (SA Explorer, 2014). Laboratory analysis of gas emissions and chemical composition of feed was conducted at the University of Zululand, KwaDlangezwa campus in KwaZulu-Natal Province of South Africa. The University of Zululand is located within the latitude of -28°51’16” and a longitude of 31°50’45”, with an altitude of approximately 121m. The climate is subtropical with average temperatures of 28.4°C and 14.5°C in the summer and winter, respectively. The average rainfall varies from 670mm to 970mm per year experiencing summer rainfalls from October to March (SA Explorer, 2014).

4.2.2 Experimental animals and housing

A total of 50 Large White Landrace X Duroc piglets (25 gilts and 25 boars) weaned at 31 days of age with average body mass of 7kg housed in five different pens, were used. The pigs were ear-tagged for identification purposes. Pigs were housed in an open house with sufficient shade, concrete floors were partially slatted, allowing faeces and urine to fall through a pit for collection. Pens measuring 1.5x1.0m each were fitted with a plastic tube feeder (Big Dutchman Lean Machine, Vechta, and Lower Saxony, Germany) and a low-pressure nipple drinker providing water ad libitum. Feeders were adjusted such that the feed is offered ad libitum. Temperature of the pig house was well regulated at 23°C using an automated heating and ventilation system.

4.2.3 Animal Feeding and care

A total of 50 Large White Landrace X Duroc piglets were received after weaning at 31 days of age with average body weight of 7kg. After vaccinating, weighing and tagging the piglets were placed in five different pens and fed starter for 4 weeks, grower for 6 weeks and finisher for 12 weeks. Feeders and drinkers were designed to be offered ad libitum throughout the day. The feeds (starter, grower and finisher) were formulated from the following ingredients; wheat, Soya, Potato protein, barley, triticale, and maize.

4.2.4 Chemical composition of feeds

4.2.4.1 Dry Matter and moisture content of feed

The dry matter and moisture content of the feeds (starter, grower and finisher) that were used to feed the pigs was determined as previously described in chapter three section 3.2.4.1.

4.2.4.2 Ash content

Ash content was determined according to AOAC International (2002), AOAC Official Method 942.05 as previously described in chapter three section 3.2.4.2.

4.2.5 Fibre content of feed

4.2.5.1 Neutral detergent fiber (NDF)

Neutral detergent fibre was determined as previously described in chapter three section 3.2.5.1.

4.2.5.2 Acid detergent fiber

NDF residue was used to determine acid detergent fiber (ADF) as described by Van Soest et al. (1991) using the ANKOM 220 fibre analyser (Ankom Technology, Fairport, New York). The procedure that was used is previously described in chapter three section 3.2.5.2.

4.2.5.3 Crude Protein (CP)

CP was determined according to the Kjeldahl method (Horwitz, 2000), AOAC Official Method Number 954.01 as previously described in chapter three section 3.2.5.3.

4.2.5.6 Non-polar extracts (NPE)

Hemi-cellulose was determined according to Van Soest et al. (1991) method. Hemi-cellulose was determined by subtracting ADF from NDF.

4.2.6 Growth and performance of Pig and feedlot cattle

4.2.6.1 Feed intake

The amount of feed the pigs consume was measured every day. The amount of feed was measured in grams per pig. Feed intake in each replicate was measured using the following formula:

Feed intake (FI) = feed provided (kg) – feed left over (kg)

4.2.6.2 Weight gain

The animal pig weight was measured every time the animal moved from one feeding ration to another to monitor their performance. The overall body weight gained was determined by subtracting the initial body weight from the final body weight.

4.2.6.3 Feed conversion ratio

Feed conversion ratio is a relationship between feed consumed and muscle deposition.

The FCR was measured every time the animal moved from one feeding stage to another with a formula:

$$FCR = \frac{\text{Total quantity of feed consumed per animal in kg}}{\text{Average body weight}}$$

4.2.7 Preparation of effective microorganism

4.2.7.1 Effective microorganism solution

Effective microorganism's solution was obtained from Efficient Microbes in Durban, South Africa. The solution is called Super EM which is a water-soluble liquid concentration of probiotic microorganisms (Probio, 2016). Super EM is an inactive solution, which can be activated by adding water. The EM was activated by adding water in different concentrations (EM0%, EM10%; EM20% and EM30%,) before using to spray the cattle faeces for odour control. Each treatment had three (3) replicates for accuracy purposes.

4.2.8 Faecal sampling

4.2.8.1 Preparation of samples for odour measurements

Swine faecal samples for treatment with EM were collected from animals fed with starter, grower and finisher pens. Fresh faecal samples were collected randomly from 20 different faecal deposits

in the starter pen mixed thoroughly before using to make 16 samples of 300g each in a 1L glass beaker (79mm x 39.5mm radius). These samples were used to form four groups of four replicates for treatment with EM. The activated EM solution at different concentrations (0% EM (Control); 10% EM; 20% EM; and 30% EM) were used to spray the different groups. The same faecal procedure and treatment was repeated when the animals moved to the grower and finisher pens. The height of the samples (20cm) in the beaker was similar to the height of cattle faecal deposits as observed in the feedlot natural environment. The samples were treated with EM by spraying at different concentrations and gaseous emission captured in traps (odour measured) once a week for four weeks. The samples were kept in a well-ventilated room and 2 m apart at the university farm house during experimentation.

4.2.9 Odour sampling

Pig odour sampling was conducted as previously described in chapter 3 section 3.2.9.

4.2.10 GC-MS analysis of prepared samples and compound identification

Volatile samples from swine were analysed using a coupled Varian 3800 gas chromatograph (Varian Palo Alto, CA, USA) and Varian 1200 mass spectrometer. The gas chromatograph was equipped with an Alltech EC–WAX column of 30 m × 0.32 mm internal diameter × 0.25 mm film thickness (Alltech Associates Inc.,Deerfield, IL, USA). Helium was used as the carrier gas at a flow rate of 1 mL min^{-1}. The traps were placed in a Varian 1079 injector by means of a Chromatoprobe fitting and thermally desorbed by heating the injector at 40 °C for 2 min with a 20:1 split ratio and then increased to 200 °C, and then held at 200 °C min-1 in splitless mode for thermal desorption. After a 3 min hold at 40 °C, the gas chromatograph oven was ramped up to 240 °C at 10 °C min-1 and held there for 12 min. Compound identification was carried out using the Varian Workstation software with NIST05 mass spectral library and comparisons with

retention times of chemical standards, where available, as well as comparisons between calculated Kovats retention indices and those published in the literature. A homologous series of alkanes (C8–C20) was used to determine Kovats retention indices. Compounds were verified using retention times of authentic standards (97–99.5% Sigma Aldrich Inc. GmbH, Germany), and (3E)-1, 3-octadiene (98 %, ChemSampco, USA), and published Kovats indices. Compounds present at higher or similar percentages in controls were considered as contaminants and excluded from the analysis. For quantification of emission rates, known amounts of standards of dominant compounds were injected into cartridges and thermally desorbed under identical conditions to the samples. The peak areas of compounds in the samples were compared with those of the standards and used to calculate the total emission rates of compounds per faecal sample per hour and emission rate per compound per faecal sample per hour. Individual compounds comprising ≥10% of the average relative amount that were emitted in almost all growth stages and during different feeding rations were considered dominant compounds.

4.2.11 Statistical analysis

All data was analysed using the statistical package of social science (SPSS). The means of chemical components of the different diets (DM, OM, Ash, Fat, MC, NDF, ADF, HEM, CP, Ca, Mg, K, Na; P, Zn, Cu, Mn, and Fe) and , performance parameters (weight gain, feed conversion ratio, and feed intake) were analysed using an analysis of variance (ANOVA) under statistical package of social science. Tukey's test was used for comparison between means of chemical components and performance parameters which were different when $P<0.05$.

For odour analysis, Primer 6 program (Clarke and Gorley, 2006) was used to analyse and compare scent profiles emitted from manure of swine fed with different diets (starter, grower, and finisher). Nonmetricmultidimensional scaling (NMDS), based on Bray–Curtis similarities of square root

transformed data, was used to detect similarities among samples. To evaluate how well or poorly the particular configuration reproduces the observed distance matrix, the stress value is given. The smaller the stress value, the better the fit of the reproduced ordination to the observed in the distance matrix (Clarke, 1993). Differences in scent profiles between different feeding stages was assessed by ANOSIM (Clarke and Gorley, 2006) with 10 000 random permutations.

4.3 Results

4.3.1 Chemical composition of feeds

There were significant differences ($P<0.05$) in all components measured across all feed varieties apart from calcium, magnesium, phosphorus and manganese (Table 4.1). The highest ($P<0.05$) dry matter content was observed in starter feed. Grower had the highest fibre content (NDF, ADF and hemicellulose). The starter was noted for the highest crude protein content while there was no significant difference ($P>0.05$) between the grower and finisher crude protein levels.

Table 4.1 Chemical composition of swine diets.

Components	**Feed type**				
	Starter	**Grower**	**Finisher**	**SEM**	**P-value**
DM (%)	93.4^{c}	91.21^{b}	74.60^{a}	3.75	0.05
MC (%)	6.27^{a}	8.98^{b}	25.26^{c}	3.75	0.05
Ash (%)	5.57^{b}	4.85^{a}	4.77^{a}	0.16	0.05
Fat (%)	6.47^{b}	2.44^{a}	2.49^{a}	0.85	0.05
OM (%)	94.43^{a}	95.15^{b}	95.23^{b}	0.16	0.05
NDF (%)	20.34^{a}	22.55^{c}	21.47^{b}	0.41	0.05
ADF (%)	2.86^{b}	3.98^{b}	2.23^{a}	0.35	0.05
HEM(%)	16.48^{a}	18.66^{b}	19.24^{b}	0.54	0.05
CP (%)	20.31^{b}	16.86^{a}	16.45^{a}	0.78	0.05
Ca (%)	0.60^{a}	0.66^{a}	0.68^{a}	0.02	N
Mg (%)	0.28^{a}	0.23^{a}	0.21^{a}	0.01	N
K (%)	0.86^{b}	0.79^{ab}	0.76^{a}	0.02	0.05
Na (%)	0.34^{b}	0.19^{a}	0.21^{a}	0.03	0.05
K/Ca+Mg(%)	0.48^{a}	0.39^{a}	0.38^{a}	0.02	N

P	**(%)**	0.61[a]	0.59[a]	0.59[a]	0.01	N
Zn	**(ppm)**	2038.29[b]	207.88[a]	135.22[a]	394.63	0.05
Cu	**(ppm)**	186.74[c]	138.69[a]	168.60[b]	8.95	0.05
Mn	**(ppm)**	96.49[a]	71.18[a]	64.58[a]	7.66	N
Fe	**(ppm)**	499.88b	273.97[a]	221.24[a]	56.99	0.05

DM= Dry matter; MC= Moisture content; OM= Organic matter; NDF=Nutrient detergent fibre; ADF= Acid detergent fiber; HEM= Hemicellulose; CP= Crude protein; Ca= Calcium; Mg= Magnesium; K= Potassium; Na= Sodium; P= Phosphorus; Zn= Zinc; Cu= Copper; Mn= Manganese; Fe= Iron, SEM= Standard error of means; N= No significant difference. Means within the same raw with different subscripts are significantly different (P<0.05).

4.3.2 Growth and performance of Pig

There were significant differences (P<0.05) in feed intake, weight gain and feed conversion ratio from pigs feed with all the different diets (Starter, grower and finisher). The feed intake and feed conversion ratio increased as the animal moved from one diet to another. The weight gain increased when the animal moved from starter to grower. However, it decreased once the animal changed diet from grower to finisher (Table 4.2).

Table 4.2. Swine feed intake weight gain and feed conversion ratio.

Components	**Feed type**				
	Starter	**Grower**	**Finisher**	**SEM**	**P-value**
Feed intake (Kg/pig/day)	0.55[a]	3.03[c]	6.55[b]	1.43	N
Weight gain (Kg/pig/day)	0.89[a]	1.92[c]	1.42[b]	0.19	0.05
Feed conversion ratio	0.57[a]	1.58[c]	4.66[b]	1.02	N

SEM; Standard error of means. Means within the same raw with different subscripts are significantly different (P<0.05).

4.3.3 Effect of EM on gaseous emissions from manure of swine fed with starter diet.

A total of 41 VOCs were emitted from swine litter that was fed starter feed ration (Table 4.3.) This consisted of 5 alcohols, 5 aldehydes, 13 volatile fatty acids (VFA), 2 ketones, 7 Phenolics, 2 Terpenoids, 1 Amide, 3 Sulphur compounds and 3 nitrogen compounds (Table 4.3a, b, c, d and e). The quantity and quality of compounds emitted during week-3 fermentation process of the manure varied (P<0.05). After the first week of fermentation and treatment of samples with EM at different concentrations [ST0 (10% EM); ST1 (20% EM); ST2 (30% EM)], 30 compounds were detected.

The most dominant compounds that were emitted in high quantity from the control were; VFAs (propanoic acid, butanoic acid, isovaleric acid and pentanoic acid); Phenolics (phenol and 4-methyl phenol); Sulphur compounds (dimethyl disulphide and dimethyl trisulphide) and Nitrogen compounds (2-piperidinone; indole; and skatole). Treatment ST3 resulted in high reduction ($P<0.05$) of all dominant VFAs, phenol, 4-methyl phenol, dimethyl trisulphide and all nitrogen compounds. However, ST2 and ST3 treatments in week 1 resulted to increased butanoic acid, isovaleric acid, pentanoic acid, phenylethyl alcohol and dimethyl disulphide than in the controls.

A further decrease in the quantity of the VOCs was observed in the 2nd week, 25 compounds were identified, which was less than in the first week. 1-butanol (Alcohol) and dimethyl disulphide (Sulphur compound) was observed in higher concentration in treatment ST3 compared to the control. However, it was further mitigated to very lower concentrations in week 3. The most dominant odour compounds was dimethyl disulphide which was higher in the control but undetectable after EM treatment at all 3 levels.

4.3.4 Effect of EM on gaseous emissions from manure of swine fed with grower diet.

Faecal samples from pigs fed with grower diet resulted in the emission of 40 compounds which are represented in Table 4.4. These compounds included 4 alcohols, 5 aldehydes, 14 VFAs, 2 ketones, 7 phenolics, 1 terpenoid, 1 amide, 3 sulphur compounds and 3 nitrogen compounds. Volatile fatty acids (Acetic acid, propanoic acid, Isobutyric acid, butanoic acid, isovaleric acid and pentanoic acid), phenolics (Phenol, 4-methyl phenol and 3-methyl-phenol), sulphur-compounds (Dimethyl disulphide and dimethyl trisulphide) and N-compounds (2-piperidinone, indole and skatole) were the dominant compounds in week 1. Treatment GT2 and GT3 resulted in reduction of all of the dominant Sulphur-compounds and N-compounds to lower concentrations compared to those in the control. Treatment GT2 and GT3 on faecal samples resulted in a complete mitigation

of propanoic acid, isobutyric acid, butanoic acid, 2-piperidinone and indole to 0% when compared to control emissions in week 2. Week 2 also resulted in a decrease in the total quantity of compounds emitted (27) but some of the compounds (1-butanol, isovaleric acid, pentanoic acid, 4-methyl phenol, dimethyl disulphide, dimethyl trisulphide and skatole) were still relatively high in concentrations. However, treatment GT3 mitigated isovaleric acid and pentanoic acid to lower concentrations.

Aldehydes (Heptanal, octanal, nonanal, and decanal) increased in all treatments (mostly in GT3 treatment) while all the odourous compounds (VFAs, phenolis, sulphur and nitrogen compounds) decreased in week 3. However, only dimethyl disulphide remained in high concentration than the control.

4.3.5 Effect of EM on gaseous emissions from manure of swine fed with finisher diet

The odour compounds (VOCs) emitted by faecal samples from swine fed finisher feed ration are summarized in Table 4.5. In total, 38 compounds were detected, which compromises of 3 alcohols, 5 aldehydes, 14 VFAs, 2 ketones, 7 Phenolics, 1 Terpenoids, 1 Amide, 2 Sulphur compounds and 3 nitrogen compounds (Table 4.5a,b,c,d and e). After EM treatment in week 1, a total of 36 gasses were detected with isovaleric acid, pentanoic acid, 4-methyl phenol, indole and skatole as the most dominant compounds but were significantly reduced ($P<0.05$) by treatment FT3. On the other hand, dimethyl disulphide was on the increase in treatment FT3. A reduction of the number and concentration of compounds in week 2 was observed with a total of 24 compounds identified. This reduction was accounted for by the total mitigation of odorous compounds, such as Propanoic acid, phenol, 4-ethyl phenol, 3-ethyl phenol, butanamide, 2-piperidinone and indole to 0% in treatment FT2 and FT3. Acetic acid and 1-butanol was observed to have increased in FT3. In week 3 a further reduction of VOCs was observed, where only 20 compounds were detected. Except for

Hexadecanoic acid and 3-methyl-phenol, no VFAs, phenolic and nitrogen compounds were emitted from samples FT3. Dimethyl disulphide and dimethyl trisulphide were the only dominant odorous compounds identified although they were in low concentration in treatment FT3. There was an increase of nonanal and decanal in FT3 when compared to the control.

4.3.6 Summary statistics and level of significance for odour emission

A two way ANOSIM tests for differences on odour emission using weeks as a factor (Across all treatment groups) showed that there was a significant difference between week 1 and week 2 ($R=0.614$; $P<0.05$); week 1 and week 3 ($R=0.536$; $P<0.05$); and week 2 and week 3 ($R=0.25$; $P=0.01$). The test for differences in treatment groups (across feed type groups) indicated that there was no significant difference (global $R=0.059$; $P>0.05$. Furthermore, the test for differences on odour emission using feed type as a factor (Across all week groups) demonstrated that there was no significant difference between starter and grower ($R=0.085$; $P>0.05$) and there was no significant difference between grower and finisher ($R=0.06$; $P>0.05$). However, there was a significant difference between starter and finisher ($R=0.157$; $P<0.05$).

A two way simper analysis using weeks as a factor (Across all feed groups) indicated that 2-piperidinone, 3-Methylpentanoic acid, pentanoic acid, butanamide, dimethyl disulphide, decanal, dimethyl trisulphide, phenylethyl alcohol, isovaleric acid, 1-Octanol and 1-butanol characterized the emissions of samples across the different feeds and accounts for 82% of average similarity in week 1. On the other hand, dimethyl disulphide, 1-butanol, dimethyl trisulphide, octanal, heptanal and 1-Octanol accounted for 81% average similarity in week 2. Furthermore, in week 3 dimethyl disulphide, 3-Octanol, nonanal, octanal, 1-butanol, and heptanal accounted for 84% average similarity of total emissions.

Table 4.3a. Effect of EM treatments on volatile organic compounds (Alcohol and Aldehyde) concentrations from pig manure in relation to feed diet over a three week period.

COMPOUND CLASS		ALCOHOL					ALDEHYDE				
		1-butanol	1-Pentanol	1-hexanol	3-Octanol	1-Octanol	Hexanal	Heptanal	Octanal	Nonanal	Decanal
	KRI	1141	1255	1352	1407	1582	1090	1180	1278	1388	1531
	CAS	71-36-3	111-70-6	111-27-3	589-98-0	111-87-5	66-25-1	111-71-7	124-13-0	124-19-6	112-31-2
W1	ST0	2.32	-	-	-	-	-	-	0.21	17.20	-
	ST1	0.03	-	-	-	-	0.01	-	0.54	0.07	-
	ST2	2.62	0.04	-	-	-	-	0.13	0.11	0.11	-
	ST3	1.59	-	-	-	-	-	0.59	0.17	0.18	-
W2	ST0	9.91	-	-	-	-	-	0.43	0.96	0.72	-
	ST1	8.82	-	-	-	-	-	10.49	10.34	7.68	-
	ST2	4.50	-	-	-	0.11	-	0.57	0.60	0.75	-
	ST3	35.74	-	-	-	-	-	4.98	5.14	2.81	-
W3	ST0	3.18	-	-	-	-	-	6.38	6.26	10.67	0.96
	ST1	0.13	-	0.04	0.04	-	0.12	0.15	0.15	20.62	10.95
	ST2	4.88	-	-	-	-	1.37	2.12	3.84	2.92	1.58
	ST3	3.00	-	0.02	-	-	4.51	5.56	8.62	-	1.46
W1	GT0	7.85	2.25	-	-	-	-	0.11	7.41	1.44	-
	GT1	4.28	0.16	0.09	-	0.04	0.37	0.21	3.03	0.28	-
	GT2	1.56	0.23	0.04	-	1.07	1.11	0.16	-	0.33	-
	GT3	2.54	0.55	-	-	-	-	-	0.34	-	-
W2	GT0	14.31	-	-	-	-	-	3.53	4.54	6.48	-
	GT1	12.61	-	-	-	-	-	5.45	1.35	3.28	-
	GT2	4.49	-	-	-	-	-	-	0.52	-	-
	GT3	15.61	-	-	-	-	-	1.57	3.31	-	-
W3	GT0	2.24	-	-	-	0.51	-	3.71	3.13	5.18	0.95
	GT1	4.11	-	-	-	0.89	0.08	39.26	8.33	10.69	3.26
	GT2	4.97	-	-	-	-	2.85	18.63	4.17	5.27	1.55
	GT3	-	-	-	-	-	-	4.35	8.08	13.95	35.24
W1	FT0	2.18	-	-	-	-	-	0.47	2.03	1.66	-
	FT1	1.05	31.51	0.13	-	-	-	-	0.18	0.58	-
	FT2	2.61	0.47	0.23	-	-	-	-	-	0.10	-
	FT3	0.11	-	-	-	-	0.02	0.11	1.49	0.64	-
W2	FTO	52.93	-	-	-	-	2.51	3.42	5.68	11.68	1.88
	FT1	35.02	-	-	-	-	-	1.98	5.31	0.65	0.30
	FT2	7.77	-	-	-	-	-	1.57	2.07	2.03	0.04
	F3	17.78	-	-	-	-	-	2.80	8.76	4.19	0.43
W3	FT0	38.35	-	-	-	-	0.42	4.12	4.03	4.32	8.06
	FT1	8.10	-	-	-	-	-	1.97	6.02	17.95	4.74
	FT2	0.68	-	-	-	-	-	-	0.87	7.62	3.34
	FT3	13.32	-	-	-	-	-	6.98	8.04	27.97	18.17

ST0= Starter treatment control (0% EM); ST1= Starter treatment 1 (10% EM); ST2= Starter treatment 2 (20% EM); ST3= Starter treatment 3 (30% EM); GT0= Grower treatment 0 control (0% EM); GT1= Grower treatment 1 (10% EM); GT2= Grower treatment 2 (20% EM); GT3= Grower treatment 3 (30% EM); FT0= Finisher treatment 0 control (0% EM); FT1= Finisher treatment 1 (10% EM); FT2= Finisher treatment 2 (20% EM); FT3= Finisher treatment 3 (30% EM); W1= sampling on the first week; W2= sampling after 2 weeks; W3= sampling after 3 weeks. Compounds were identified by common names and CAS (Chemical Abstract Service) registry number, and listed according to estimated Kovats retention index (KRI) within each compound class. Values are mean percentages of the total peak area.

Table 4.3b. Effect of EM treatments on volatile organic compounds (Volatile fatty acids) concentrations from pig manure in relation to feed diet over a three week period.

COMPOUND CLASS		VOLATILE FATTY ACIDS													
		Acetic A	Propanoic A	Isobutyric A	Butanoic A	Isovaleric A	3-Methylpentanoic A	Pentanoic acid	Isocrotonic A	4-methyl-pentanoic A	Hexanoic acid	Heptanoic A	Octanoic A	Nonanoic A	Dodecanoic A
	KRI	1458	1537	1604	1645	1678	1681	1758	1773	1844	1885	1952	2094	2197	2454
	CAS	64-19-7	79-09-4	79-31-2	107-92-6	503-74-2	105-43-1	109-52-4	503-64-0	646-07-1	142-62-1	111-14-8	124-07-2	112-05-0	143-07-7
W1	ST0	0.63	1.03	0.57	3.06	1.90	-	0.01	-	2.83	1.21	-	-	-	-
	ST1	2.54	2.04	0.27	13.87	5.17	-	11.58	-	3.89	5.55	-	0.01	-	-
	ST2	3.21	5.04	1.07	41.31	20.32	-	12.69	-	0.13	3.99	-	-	-	-
	ST3	1.34	2.08	1.28	4.05	4.62	0.14	2.89	-	0.11	0.28	-	-	-	-
W2	ST0	0.05	-	29.21	0.18	0.29	-	0.44	-	-	0.15	0.02	0.03	-	-
	ST1	-	-	-	0.58	9.27	-	0.24	-	-	-	-	-	-	-
	ST2	-	-	-	-	-	-	0.01	-	-	-	-	0.02	-	-
	ST3	0.15	-	-	0.06	0.03	-	0.72	-	-	1.73	-	0.03	0.55	-
W3	ST0	7.97	0.37	-	1.16	1.27	-	1.43	-	-	0.84	0.13	0.19	0.05	0.10
	ST1	2.06	-	0.01	32.39	0.02	-	0.07	-	-	0.06	-	-	-	0.01
	ST2	0.59	0.08	0.17	0.60	2.35	-	1.29	-	-	21.12	0.02	0.01	-	0.04
	ST3	0.30	-	0.02	0.06	1.91	-	0.09	-	-	0.11	29.32	0.03	-	0.01
W1	GT0	3.94	7.14	2.48	8.20	4.32	6.06	3.16	-	0.02	0.99	0.01	-	-	-
	GT1	2.46	1.76	2.81	10.34	7.65	-	12.82	-	0.08	2.10	-	-	-	-
	GT2	1.38	12.89	2.13	26.22	4.68	-	23.10	0.03	3.82	5.04	0.13	0.07	-	-
	GT3	1.08	1.76	1.76	17.88	-	-	19.03	-	0.46	9.52	0.06	0.03	-	-
W2	GT0	0.49	0.20	0.63	2.63	4.29	-	2.29	-	0.51	1.51	-	0.23	-	-
	GT1	19.10	-	1.01	0.25	0.64	-	0.30	-	-	0.19	-	0.44	0.88	-
	GT2	2.13	-	-	-	5.39	-	0.64	-	0.10	0.38	-	-	-	-
	GT3	5.07	-	-	-	0.50	-	0.26	-	-	-	-	0.31	0.61	-
W3	GT0	5.56	-	-	0.67	0.43	-	33.37	-	-	0.21	0.01	0.01	-	0.24
	GT1	-	-	-	-	1.76	-	-	-	1.03	0.25	-	0.02	-	0.36
	GT2	-	-	0.49	-	5.61	-	1.84	-	1.26	-	-	-	-	-
	GT3	-	-	-	-	0.10	-	0.07	-	0.14	0.03	-	0.01	-	-
W1	FT0	4.20	-	3.04	1.65	14.03	5.70	15.87	-	0.75	0.62	-	0.45	-	-
	FT1	0.49	0.68	1.67	1.39	15.33	-	8.53	0.01	2.19	3.11	-	-	-	-
	FT2	1.15	-	2.92	1.44	17.73	-	30.64	0.42	0.71	0.19	3.84	-	-	-
	FT3	2.70	2.34	1.86	6.52	5.61	-	8.33	-	0.67	7.54	0.63	0.07	-	-
W2	FTO	1.05	0.32	-	0.78	-	-	0.30	-	-	-	-	-	-	-
	FT1	7.31	-	0.57	0.16	3.14	-	1.03	-	0.16	0.16	-	0.67	0.27	-
	FT2	6.33	0.08	0.01	0.10	0.95	-	0.15	-	0.01	0.50	-	0.05	-	-
	F3	15.68	-	0.34	1.20	2.01	-	2.42	-	0.32	1.77	0.31	0.46	0.42	-
W3	FT0	2.07	-	-	-	-	-	-	-	-	-	-	-	-	-
	FT1	0.05	-	-	-	0.29	-	-	-	-	-	-	-	-	-
	FT2	0.07	-	0.01	-	-	-	-	-	-	-	-	23.46	0.02	0.01
	FT3	-	-	-	-	-	-	-	-	-	-	-	-	-	-

ST0= Starter treatment control (0% EM); ST1= Starter treatment 1 (10% EM); ST2= Starter treatment 2 (20% EM); ST3= Starter treatment 3 (30% EM); GT0= Grower treatment control (0% EM); GT1= Grower treatment 1 (10% EM); GT2= Grower treatment 2 (20% EM); GT3= Grower treatment 3 (30% EM); FT0= Finisher treatment control (0% EM); FT1= Finisher treatment 1 (10% EM); FT2= Finisher treatment 2 (20% EM); FT3= Finisher treatment 3 (30% EM); A= Acid; W1= sampling on the first week; W2= sampling after 2 weeks; W3= sampling after 3 weeks. Compounds were identified by common names and CAS (Chemical Abstract Service) registry number, and listed according to estimated Kovats retention index (KRI) within each compound class. Values are mean percentages of the total peak area.

Table 4.3c. Effect of EM treatments on volatile organic compounds (Ketones, furanoids and terpenoids) concentrations from pig manure in relation to feed diet over a three week period.

COMPOUND CLASS		KETONES		FURANOIDS		TERPENOIDS	
		2-Heptanone	3-Octanone	2-pentyl-furan	Hydroxymethylfurfural	Verbenone	α-Isophoron
	KRI	1198	1249	1220	2471	1428	1607
	CAS	110-43-0	106-68-3	3777-69-3	67-47-0	80-57-9	78-59-1
W1	ST0	-	0.07	-	-	-	-
	ST1	-	0.15	-	-	-	-
	ST2	-	-	-	-	-	-
	ST3	-	-	-	-	-	-
W2	ST0	-	-	-	-	-	-
	ST1	-	1.02	-	3.25	-	-
	ST2	-	0.27	-	-	-	-
	ST3	-	-	-	-	-	-
W3	ST0	-	-	-	-	-	-
	ST1	-	-	32.60	-	-	-
	ST2	0.08	-	0.23	-	3.33	0.21
	ST3	-	-	0.07	-	-	31.44
W1	GT0	9.48	-	-	-	-	-
	GT1	-	2.37	-	-	-	-
	GT2	-	-	-	-	0.02	-
	GT3	-	0.09	-	-	0.73	-
W2	GT0	-	-	-	-	-	-
	GT1	-	1.36	-	-	-	-
	GT2	-	-	-	-	-	-
	GT3	-	-	-	-	-	-
W3	GT0	-	-	-	-	-	-
	GT1	-	-	-	-	-	-
	GT2	-	-	-	-	-	-
	GT3	-	-	-	-	-	-
W1	FT0	2.37	-	-	-	-	-
	FT1	0.62	0.14	-	-	-	-
	FT2	-	-	-	-	-	-
	FT3	-	-	-	-	-	-
W2	FTO	-	-	-	-	-	-
	FT1	-	-	-	-	-	-
	FT2	-	-	-	-	-	-
	F3	-	-	-	-	-	-
W3	FT0	-	-	-	-	8.07	-
	FT1	-	-	-	-	-	-
	FT2	-	-	-	-	-	-
	FT3	-	-	-	-	8.01	-

ST0= Starter treatment control (0% EM); ST1= Starter treatment 1 (10% EM); ST2= Starter treatment 2 (20% EM); ST3= Starter treatment 3 (30% EM); GT0= Grower treatment control (0% EM); GT1= Grower treatment 1 (10% EM); GT2= Grower treatment 2 (20% EM); GT3= Grower treatment 3 (30% EM); FT0= Finisher treatment control (0% EM); FT1= Finisher treatment 1 (10% EM); FT2= Finisher treatment 2 (20% EM); FT3= Finisher treatment 3 (30% EM); W1= sampling on the first week; W2= sampling after 2 weeks; W3= sampling after 3 weeks. Compounds were identified by common names and CAS (Chemical Abstract Service) registry number, and listed according to estimated Kovats retention index (KRI) within each compound class. Values are mean percentages of the total peak area.

Table 4.3d. Effect of EM treatments on volatile organic compounds (Phenolics and Amide) concentrations from pig manure in relation to feed diet over a three-week period.

COMPOUND CLASS		PHENOLIC						AMIDE
		Phenylethyl alcohol	Phenol	4-methyl phenol	3-methyl-phenol	4-ethyl phenol	3-Ethyl phenol	butanamide
	KRI	1937	2043	2076	2080	2079	2167	1887
	CAS	60-12-8	108-95-2	106-44-5	108-39-4	123-07-9	620-17-7	541-35-5
W1	ST0	0.42	0.93	31.77	0.51	0.34	0.05	0.03
	ST1	0.55	0.76	15.71	-	0.72	-	0.04
	ST2	0.74	0.09	3.97	2.10	0.14	-	-
	ST3	30.78	0.34	12.58	-	0.44	-	-
W2	ST0	23.05	0.06	0.12	1.16	0.54	-	-
	ST1	0.16	0.34	7.51	0.43	-	-	-
	ST2	0.82	0.12	3.36	0.35	0.12	-	-
	ST3	-	0.31	0.71	-	-	-	-
W3	ST0	-	-	0.81	-	1.06	-	-
	ST1	-	-	-	0.01	-	-	-
	ST2	-	0.06	-	0.19	0.38	-	-
	ST3	0.14	0.05	0.35	-	0.09	-	-
W1	GT0	0.17	0.60	12.18	1.80	0.20	-	-
	GT1	0.17	1.12	23.43	-	0.35	0.35	12.73
	GT2	0.04	0.18	8.09	-	0.14	-	0.03
	GT3	0.10	0.36	14.96	-	0.32	0.01	0.03
W2	GT0	0.04	0.72	3.79	2.03	0.12	0.17	0.04
	GT1	-	-	2.38	-	0.11	-	-
	GT2	0.51	0.61	0.37	14.30	-	-	-
	GT3	0.56	-	13.34	3.79	1.23	-	-
W3	GT0	-	0.02	0.75	-	-	-	-
	GT1	-	0.09	0.46	-	-	-	-
	GT2	0.07	0.22	1.36	0.18	0.31	-	-
	GT3	-	0.01	0.33	0.03	0.01	-	-
W1	FT0	0.14	0.85	27.91	-	0.52	0.54	0.15
	FT1	0.20	1.15	22.74	0.29	1.01	0.26	0.07
	FT2	0.02	0.22	8.02	-	0.03	0.21	-
	FT3	0.18	0.92	16.60	-	0.70	-	0.06
W2	FTO	-	0.10	2.25	-	-	-	-
	FT1	-	-	-	-	-	-	-
	FT2	-	-	0.15	0.18	-	-	-
	F3	-	-	-	0.88	-	-	-
W3	FT0	-	-	-	-	-	-	-
	FT1	-	0.44	-	-	-	-	-
	FT2	-	9.38	0.10	-	-	-	-
	FT3	-	-	-	1.04	-	-	-

ST0= Starter treatment control (0% EM); ST1= Starter treatment 1 (10% EM); ST2= Starter treatment 2 (20% EM); ST3= Starter treatment 3 (30% EM); GT0= Grower treatment control (0% EM); GT1= Grower treatment 1 (10% EM); GT2= Grower treatment 2 (20% EM); GT3= Grower treatment 3 (30% EM); FT0= Finisher treatment control (0% EM); FT1= Finisher treatment 1 (10% EM); FT2= Finisher treatment 2 (20% EM); FT3= Finisher treatment 3 (30% EM); W1= sampling on the first week; W2= sampling after 2 weeks; W3= sampling after 3 weeks. Compounds were identified by common names and CAS (Chemical Abstract Service) registry number, and listed according to estimated Kovats retention index (KRI) within each compound class. Values are mean percentages of the total peak area.

Table 4.3e. Effect of EM treatments on volatile organic compounds (Sulphur and nitrogen compounds) concentrations from pig manure in relation to feed diet over a three week period.

COMPOUND CIASS		SULPHUR COMPOUNDS			NITROGEN COMPOUNDS		
		Dimethyl disulphide	Dimethyl trisulphide	Dimethyl sulfone	2-Piperidinone	Indole	Skatole
	KRI	1074	1376	1941	2127	2421	2459
	CAS	624-92-0	3658-80-8	67-71-0	675-20-7	120-72-9	83-34-1
W1	ST0	27.99	5.97	-	0.08	0.03	0.16
	ST1	30.76	4.14	-	0.42	0.06	0.20
	ST2	1.66	0.42	-	0.03	0.01	0.03
	ST3	33.60	2.18	-	0.06	0.01	0.08
W2	ST0	30.97	0.95	-	-	-	0.18
	ST1	27.63	2.38	-	-	-	0.75
	ST2	84.05	2.41	-	-	-	0.12
	ST3	42.70	1.67	-	-	-	-
W3	ST0	40.04	14.58	-	-	-	-
	ST1	0.35	0.02	-	-	-	0.01
	ST2	12.33	9.18	0.02	-	-	-
	ST3	1.64	1.97	0.02	-	-	-
W1	GT0	7.80	10.37	-	-	0.03	0.10
	GT1	1.92	0.94	-	0.04	0.33	3.82
	GT2	-	1.36	-	0.06	0.02	0.07
	GT3	3.78	3.56	-	-	0.06	0.10
W2	GT0	36.34	8.05	-	0.24	-	0.51
	GT1	29.21	14.06	-	-	-	0.79
	GT2	47.35	7.66	-	-	-	0.64
	GT3	27.77	8.25	-	-	-	3.16
W3	GT0	1.14	2.73	0.20	-	-	-
	GT1	19.61	6.25	-	-	0.33	-
	GT2	35.45	14.58	-	-	-	-
	GT3	30.91	4.70	-	-	-	0.09
W1	FT0	4.68	5.18	-	-	0.14	0.86
	FT1	2.78	2.47	-	0.10	0.01	0.34
	FT2	-	0.13	-	-	-	0.07
	FT3	40.64	0.79	-	0.07	0.07	0.42
W2	FTO	8.43	2.60	-	-	-	0.19
	FT1	16.63	0.67	-	-	-	-
	FT2	34.55	7.04	-	-	-	0.55
	F3	26.21	2.95	-	-	-	0.08
W3	FT0	13.33	11.94	-	-	-	-
	FT1	44.58	11.80	-	-	0.22	-
	FT2	5.04	30.58	-	-	0.17	-
	FT3	5.26	2.69	-	-	-	-

ST0= Starter treatment control (0% EM); ST1= Starter treatment 1 (10% EM); ST2= Starter treatment 2 (20% EM); ST3= Starter treatment 3 (30% EM); GT0= Grower treatment control (0% EM); GT1= Grower treatment 1 (10% EM); GT2= Grower treatment 2 (20% EM); GT3= Grower treatment 3 (30% EM); FT0= Finisher treatment control (0% EM); FT1= Finisher treatment 1 (10% EM); FT2= Finisher treatment 2 (20% EM); FT3= Finisher treatment 3 (30% EM); W1= sampling on the first week; W2= sampling after 2 weeks; W3= sampling after 3 weeks. Compounds were identified by common names and CAS (Chemical Abstract Service) registry number, and listed according to estimated Kovats retention index (KRI) within each compound class. Values are mean percentages of the total peak area.

4.4 Discussion

The effect of diet on animal performance as well as type and quantities of VOCs emitted from pig manure agrees with what has been observed in other related studies in the literature from similar animals (Hobbs et al., 1997, Le et al., 2005, Ni et al., 2012, Mpendulo et al., 2018). Diet clearly improved performance of pig, as feed intake and weight gain increased as the animal move from one diet to the other. The feed conversion ratio also increased as the animal grew older with changing diets. The higher FCR meant that animals were not using the feed as efficiently as the younger. This was due to the fact that the quantities of fibre increased in the diet as the animal grew older. However, this also a good indicator that the diet is changing which implies the gaseous emissions from both pigs should also vary. Diet should be considered as the first step to control odour in livestock production systems. This is backed by literature that odour generation by microbial conversion depends on nutrient residues in the gut of animals and during manure storage (Le Phung et al., 2005). Proteins and fermentable carbohydrates are the main precursors of odour formation in the digestive tract of animals, in urine and in manure. The high quantities of VFAs detected in pig manure from this study agrees with the ones in a similar study done by Le et al. (2009). High amounts of VFAs may be due to high quantities of carbohydrates or fibre (NDF, Hemicellulose) content in the diet, particularly during the grower and finisher feeding phase. This is because growing pigs do not digest plant fibre well (Ratanpaul et al., 2019), so feeds with high fibre will be less digestible than those with low fibre. Therefore, most of the fibre get excreted in the faeces which are subsequently converted by the microbes in the manure to VFAs such as acetic acid, propionic and butyric acids (Le et al., 2005). *Clostridium, bacillus and Lactobacillus,* are the most dominant microbes in swine manure responsible for fermentation (Lim et al., 2018). The proportion of VFAs produced can vary, depending on the type of substrate (cellulose, hemicellulose and lignin) available, composition of anaerobic bacteria and the prevailing pH (Wenk, 2001). Looking at the high number of VFAs detected in this study particularly in all the controls, it is evident that there was more substrates and microbes for

the formation of VFAs. Apart from being formed from carbohydrates, VFAs are also produced by deamination of amino acids. Proteins are hydrolyzed into amino acids (L-glutamate, L-lyasin and L-alanine) by Peptolytic bacteria then deaminated and decarboxylated to Iso-butyric acid, Iso-valeric acid, and 2-methylbutyric acid (Le et al., 2007). It has also been reported that proteins and its metabolites are metabolic precursors for most of the main groups of odorous compounds (Yamaguchi et al., 2019). This is can be very critical when the dietary protein content is more than the protein requirement for pigs. Feeding of pigs with higher fibre can also promote this process because the fibrous content can only be digested in the hindgut by microbes. This implies the microbial population cannot be absorbed as protein hence will be eliminated as faeces which increases the protein content in the manure. This results in availability of substrates for generation of nitrous compounds or sulphurous compounds. In this study, a high number of nitrogen and sulphur compounds were detected in the manure of pigs fed with starter and grower diets. This might be due to the amino acids composition of dietary protein from the different feed rations provided, not meeting or matching the animal's requirement, hence resulting in a high by-pass of these compounds to the manure (Van Emous et al., 2019). Indole and Skatole are the nitrogen compounds that were emitted in high amounts in both the starter and grower manure. It has been reported that these compounds cause acute bovine pulmonary edema and emphysema (ABPE) in cattle and is responsible for boar taint in pigs (Deslandes et al., 2001, Tretola et al., 2019). Boar taint is an offensive odour and flavour that occurs when cooking and eating pork or pork products (Bonneau and Weiler, 2019). In this study indole and skatole were emitted in relatively higher amounts, but proper monitoring needs to be considered though it did not affect swine performance because of the feed intake, weight gain and feed conversion ratio. However, the off-odour (boar taint) given off by the meat while cooking could be offensive to consumers. This could result in the farmer losing profit or income since the produce would not be preferred by consumers. The use of EM to mitigate indole and skatole was a success since it was reduced to undetectable concentration in all treatment levels during week 3. The mechanism of its

reduction was not clear but further observation at the microbial and bimolecular level can give more answers.

P-Cresol (4-methyl phenol) and phenol were the highest sulphur compounds emitted from starter and grower manure. Interestingly, they are listed as hazardous (air pollutants) by the US Environmental Protection Agency with effects that includes; respiratory irritation, abdominal pain, vomiting, and corrosive lesions of the gastrointestinal tract in humans (Gupta, 2019). Parker et al. (2013) also reported that p-cresol is responsible for the overall odour impact from the VOCs emission hence mitigation of this gas is vital since it is toxic and odorous. In other studies, Zhang et al. (2019) observed that the main odour VOCs in the manure composting process were; trimethylamine, dimethyl sulphide (methyl sulphide), dimethyl disulphide and dimethyl trisulphide. The same gases were observed in this study, particularly dimethyl disulphide and dimethyl trisulphide. However, in some cases the mitigation of dimethyl disulphide by EM was not effective, since it was observed to be in higher concentration in the finisher manure during week 3 even when treated by EM at different concentrations. The reason behind this might be that the microbes in EM were unable to compete with the sulphur forming microbe in the manure. However, further research on alternatives methods can be explored in future to mitigate these sulphur compounds.

Understanding the mechanism of sulphur compounds production can be a big step towards the development of mitigation strategies. Sulphur compounds (Dimethyl disulphide, Dimethyl trisulphide, and Dimethyl sulfone) are formed from the breakdown of amino acids methionine, and cysteine while most nitrogen containing compounds (2-Piperidinon, Indole and skatole) and phenolic compounds (Phenylethyl alcohol, Phenol, 4-methyl phenol, 3-methyl-phenol, 4-ethyl phenol, 3-Ethyl phenol) are formed from microbial fermentation of amino acid L-tryptophan as suggested by Tangerman (2009). Therefore, dietary protein alteration should receive the first attention as an attempt to reduce odour by dietary manipulation. However, most producers will be reluctant as it may hinder their livestock performance and overall production. This is where solutions like EM can come in to play a major role

(mitigation) without altering the diet (Mafiri, 2014). There is a need, therefore, to explore alternative EM with potential for mitigation of these sulphur producing compounds.

The use of EM at different concentrations indicated that there was no significant difference between the treatment groups (across feed type groups) but for the control. However, there was a significant difference in odour emitted between week 1 and week 2, week 1 and week 3 and week 2 and week 3 in all treatments. This means that reduction of odour from manure using EM does not depend on concentration but it depends on time for the microbes to adapt, grow and multiply to mitigate odour particularly the purple non-sulphur bacteria (PNSB) as described by (Birkett, 2018). Although the difference in treatment groups is not significant, 30% dose of EM was preferred since it seems to mitigate most of the odorous compounds to lower concentrations early in week 2. In week 3 most of the compounds were undetectable in 30% treatments compared to the other treatment levels. It was associated the rapid growth and multiplication of EM at higher concentrations at 30% hence outcompeting the microbes in the manure. For example the yeast in EM has the ability to breakdown organic substrates and produce pyruvic acid through metabolism. Pyruvic acid can be used as a food source by the LAB in EM. In this way, the LAB use the metabolites of yeast to grow and multiply and produce volatile organic acids such as lactic acid which becomes a food source of PNSB and they can grow and multiply. The yeast in turn use the carbohydrates formed by these PNSB as a source of food and the microbes can multiply repeatedly (Zimmermann and Kamukuenjandje, 2008) hence recycling by-products and decreasing or mitigating odour production..

Purple non-sulphur bacteria (PNSB) are phototrophic microorganisms. These microorganisms have been on Earth before there was oxygen, meaning that their food source are gases that are considered harmful or toxic to human today (volatile organic compounds, Carbon Dioxide, Methane and Ammonia). This means that PNSB in EM consume toxins and harmful gases in its environment and release oxygen as one of its by-products (Leuenberger, 2015) which is very useful for oxidative processes. This mechanism of action does promote the growth of aerobic microbes which have been reported to promote plant decay

rather than fermentation hence a decrease in toxic volatiles or odorous emissions. Aerobic bacteria (bacteria which use oxygen) have been reported to consume the oxygen generated and excrete carbon dioxide, which the phototrophs can use as food. Most importantly, phototrophic microbes also excrete amino acids, antioxidants and other substances that enhance life which can then be used by the animal resulting in improved growth and performance (Leuenberger, 2015). If this is true, then feeding EM to animals should be looked into quite closely. This study did not feed the animals with EM because of the policies of the commercial farmers but subsequent research should look forward in feeding animals with EM.

4.5 Conclusion

From this study, it was concluded that diet type affected animal performance as seen by the increase in weight gain from starter to finisher as well as gaseous and odour emissions. P-Cresol (4-methyl phenol) and phenol are the most hazardous air pollutants identified because these are potentially toxic and odorous. Indole and skatole which are responsible for boar taint in pigs were also identified. Total litter gaseous emissions from starter (41), grower (40) to finisher (38) were decreased by EM treatment when compared to the control. However, gaseous emissions between EM treatments groups were the same which implies that EM effectiveness on pig litter depended on time rather than dose application. It was also noticed that total emission numbers decreased with weeks upon EM treatment and 30% treatment seems to mitigate most of the odorous compounds to lower concentration early in week 2, and in week 3 they were undetectable. However, dimethyl disulphide was not mitigated hence more alternative methods on how to mitigate this compound need to be explored. The following odorous compounds; Heptanal, octanal, nonanal, and decanal were emitted by the EM in week 3 but the mechanism of treatment was not known. Therefore, there is a need for further investigation of its mechanism of action.

CHAPTER FIVE: General discussions, Conclusions and Recommendations

5.1 General discussion

Feedlot cattle diets are often formulated to promote optimum growth in the shortest possible time which often defy the norm of natural pasture grazing with higher fibre. This is often achieved by increasing the energy and protein content by supplementation which have always been associated with potential increases in greenhouse gases. This is no different in the pig industry where the demands are even higher than the production. It has been reported that South Africans consume more than they produce which makes the country a net importer of pork meat (Meyer et al., 2020). This has led to nutritionists formulating diets with excess nutrients to achieve optimum growth in the shortest possible timeframe. However, feeding high excess nutrients such as protein rich diets generally increases nutrient bypass hence producing excreta rich in nutrients (i.e. nitrogen compounds, sulphur compounds, volatile fatty acids etc.). These nutrients in the manure (faeces) consequently are the substrates that are used by different microbes that are used to produce odours (VOCs). High fibre diets in pigs reduces performance, mainly because most fibre sources have high water holding capacities, hence limiting the amount of feed and nutrients that pigs can consume (Mpendulo, 2012). However, this is not the same for beef cattle as they are more adapted to high fibre diet than the pigs. Therefore, the cattle will turn to excrete less fibre per kg of faeces as waste than the pigs. However, the amount of VOCs released might not be solely dependent on the type and quantity of substrate waste but the types of microbes they harbour. The cattle manure is assumed to be more adapted with higher anaerobic fibrolytic microbes that can degrade these fibre when excreted in faeces than will be observed in pig litter. Even with the previous assumption, it is still very difficult to confirm that the cattle litter will yield more VOCs because the anaerobis is distorted by oxygen and that can have an effect on microbial activity. However, in formulating feeds for both beef and pork, the potential impact on environmental pollution through odour emission must not be ignored.

The main hypothesis tested in this study was that feed type and effective microorganism will not affect livestock (beef and swine) performance and decrease litter odour emission, respectively. In experimental chapter 3, the study was conducted to test the effect of different diets on beef performance and effect of EM in reducing the odour (VOCs) emitted from litters of the different diets. The hypothesis was rejected because the diet improved beef performance with increased feed intake, weight gain and feed conversion ratio as the animal was moving from one diet to the other (starter, grower and finisher) towards the marketing pens. The results showed that the diets were properly formulated and the animals were doing well but the bigger question was: what is the effect of the diet on litter gaseous emissions (in terms of environmental pollution)? This study found out that it resulted in different types and amounts of gases emitted from the manure based on the diet, hence the hypothesis was also rejected. Because of the huge number of gases that were observed, it was classified into five subgroups for simplicity and understanding, VFAs, Sulphur, Nitrogen, amides and phenolic compounds. In experimental chapter 4, the same study was repeated with the only difference being the diet types and the animals (Pigs) used. The diet improved the swine performance with increased feed intake, weight gain and feed conversion ratio as the animal moved from one diet to the other (starter, grower and finisher). The types of emissions were not different from what was observed in cattle litter emissions which included; VFAs, Sulphur, Nitrogen, amides and phenolic compounds. Though the type of emissions where not different from both beef and swine manure, the amount or concentration was different. Phenol and P-cresol were emitted in high amount in swine manure compared to beef manure in the first week. This is similar to the study of Parker et al. (2012) on dairy and swine operations where p-cresol was ranked behind only hydrogen sulphide in its contribution to pig farm odours. Several other compounds were also ranked highly, while ammonia made up a relatively small portion of the pig odour. Indole and skatole were only emitted in pig manure and were not detected on beef manure. This is could be because the feed that was fed to beef animals had no L-tryptophan amino acid since L-trytophan results in the production of indole and skatole (Le et al., 2004). Moreover, all beef feed had lower protein content compared to pig feed, hence less

nitrogen and sulphur compounds were being excreted in the beef manure to form indole and skatole. This may also explain why pig manure were potentially high in both nitrogen and sulphur compounds. Beef manure consisted of high volatile fatty acids compared to pig manure clearly because of the higher fibre content that was fed to beef hence the dominance of volatile acids as the main by-products when compared to the pig manure. Volatile fatty acids are mostly formed by microbial conversions of plant fibre in the large intestine and in manure under anaerobic conditions (Owens and Basalan, 2016). Plant fibre such as cellulose and hemicellulose are hydrolysed by microbial enzymes into monomers and then converted by the microbes into volatile fatty acids such as acetic, propionic, valeric acid, and butyric acids (Le Phung et al., 2005).

Overall, most of the odorous compounds (Indole, skatole, phenol, dimethyl sulphide and dimethyl trisulphide) were higher in pig manure than in cattle manure. So, can a case be made that pigs are more offensive than beef animals in terms of environmental pollution from litter emissions? In terms of the odour they produce as observed from this study, one can easily say yes even though there are many other complicating factors such as diet type, temperature, pH etc. Jang and Jung (2018) conducted a study on pig farms in South Korea and discovered that changes to the mix of proteins in the animals' diet resulted in significant effect on the concentrations of odorous compounds present in their manure. Other studies have also found that farm dust is an ideal conveyor of odorous molecules (Dunlop, 2011, Kumar and Yaashikaa, 2019), so the amount of dust kicked up at an operation may also be an important odour variable. However, it is clear that there is no single answer to the question of which animal produces the most unpleasant odours. The answers are inevitably tied to individual experiences and sensitivities. A smell that disgusts one person may be inoffensive or even pleasant to another.

Therefore, one of the most important questions will be, can EM mitigate these odorous or offensive compounds? The answer is yes because generally the different treatments of the manure with EM did mitigate some of the odours emitted from both beef and swine manure. For both animal production systems, there were significant differences in the odour emitted from week 1 to week 3. There was less

odour (Except for dimethyl sulphide and dimethyle trisulphide) emitted in week 3 compared to week 1 in all treatments than in the controls. Hence, the hypothesis was rejected. However, EM odour mitigation in livestock manure did not depend on concentration solely but time was a factor worth investigating in more detail.

5.2 Conclusions

The data represented in this thesis indicate that diet and EM had an effect on pig and beef animal performances and odour emissions, respectively. These findings provide great potential for testing and developing mitigation strategies for VOCs emissions from livestock enterprises and improve animal performance with the use of EM at low cost. Gaseous emissions from both animals were classified into; alcohols, aldehydes, volatile fatty acids, terpenoids, amides, phenolics, sulphur compounds and nitrogen containing compounds. From this study, it can be concluded that Phenol and P-cresol were emitted in high amount in swine manure compared to beef manure on the first week. Indole and skatole were only emitted in pig manure and were not detected in beef manure. Most of the odorous compounds (Indole, skatole, phenol, dimethyl sulphide and dimethyl trisulphide) were emitted higher concentration in pig manure than cattle.

5.3 Recommendations and further research

From the findings of both experimental chapter 1 (chapter 3) and 2 (chapter 4), it is evident that diet type has an effect on litter gaseous emissions because they are the major precursors for odour production. Providing high amounts of both fibre and proteins may result in excess nutrients excreted in the manure hence promoting odour emission. Therefore, feed should be formulated with a balance of both carbohydrates and protein based on animal requirements to prevent waste that may promote gaseous emission. A 10% EM should be used to treat beef manure and 30% EM should be considered when treating pig manure. Further research on how feeding EM to both pigs and cattle will influence animal performance and gas mitigation needs to be explored. The mechanism of action both at the microbial and molecular level needs to be explored for better understanding for mitigation purposes.

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