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METHANE EMISSIONS AND VOLATILE FATTY ACID PRODUCTION FROM MANURE EXCRETED BY CATTLE FED DIETS CONTAINING LIPIDS

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ABSTRACT

The experiment was carried out to investigate the effect of adding palm oil sludge in cattle diets on methane and volatile fatty acid production as well as on the microbial population of the manure when digested anaerobically. Four dietary treatments (T1, T2, T3 and T4) were formulated by the addition of palm oil sludge to a grass x legume silage at 0, 10, 20 and 30%, respectively. The percentage of methane on the total gas volume was measured with Sperian gas analyser while volatile fatty acids were measured using Spectrophotometer. Microbial population, digester pH and ambient temperature were also measured using microbial analysis method by Harrigan and McCance 1990, pH meter and thermometer, respectively. The experiment lasted for 90 days. Results showed that there were significant differences ($p<0.05$) in total gas production, methane production, acetic acid, propionic acid, butyric acid and microbial population among the different treatments. From the foregoing, it was concluded that palm oil sludge inclusion in cattle diet at 30% reduced methane production and acetic acid production while butyric acid and propionic acid production increased in cattle manure.

Key words; fermentation, manure, methane, muturu cattle, silage.

INTRODUCTION

Diets offered to animals can have a significant impact on manure (faeces and urine) chemistry and therefore on greenhouse gas emission during storage or following land application (Hristov *et al*., 2013). Manure storage may be required if animals are housed indoors or on feedlots. Ruminants grazing on pastures or rangelands tend to produce low methane due to aerobic digestion of their excreted manure.

Hustered (1993) estimated that animal manure accounts for 6 – 10% of total planetary CH_4 emissions due to human activities. The biogas produced during anaerobic digestion consists of methane (60-70%), carbon-dioxide (30-40%) trace amount of hydrogen sulphide and gaseous forms of nitrogen (Prasad and George, 2015). Reduced digestibility of dietary nutrients is expected to increase fermentable organic matter concentration in manure, which may increase methane emission. Maurice *et al*. (2009) observed that high energy feed has the potential to increase methane production from manure.

The housing system, type of manure collected, storage system, separation of solids and liquids, and their processing can all have significant impact on the animal and on the emission of greenhouse gases from manure. Mitigation options for greenhouse gas emissions from manure such as reducing the time of manure storage, aeration and stacking are generally aimed at decreasing the time allowed for microbial fermentation processes to occur before application to lands. Other effective strategies for reducing methane emissions from manure are acidification and cooling of stored manure. The maximum quantity of methane that can be produced by a volume of manure depends on animal type and diet (Masse *et al*., 2008). Palm oil sludge contains more phyto-nutrients than any other dietary oil and these nutrients are powerful antioxidants which protect the oil from oxidation (Bruce, 2007). Palm oil also contains a synergistic mix of vitamin E, vitamin k, alpha carotene, beta-carotene, lycopene and 20 other carotenes. In addition to ordinary vitamin E, it contains a super powerful form of vitamin E known as tocotrienol which makes it 60 times more potent than ordinary vitamin E (Oguntibeju *et. al*., 2009). However, the potency of palm oil sludge in reducing methane and volatile fatty acid production from manure excreted by cattle fed diets containing lipids has not been investigated.

MATERIALS AND METHODS

Location of study: The study was conducted at the Cattle Unit of the Department of Animal Science Teaching and Research Farm, University of Nigeria, Nsukka. Nsukka lies within longitude $6^{0}45^{1}$ and 7^{0}E and latitude, 7^{0}12.5'N (Offomata, 1975) and on the altitude 447m above sea level. The study area is located in the tropics with relative humidity ranging from 65 – 80% and mean daily temperature of 26.8^{0}C (Agbagha *et al*., 2000).

Experimental Diets: Four diets were formulated by adding palm oil sludge at 0, 10, 20 and 30% to basal silage diet to form four dietary treatments T1, T2, T3 and T4, respectively with T1 serving as control. Forage materials used were *Panicum maximum*, *Centrosema pubescens*, *Bracharia decumbens* and *Andropogon gayanus*. The forage materials were cut into an average size of 3 to 5 cm before they were ensiled for 21 days.

Experimental Animals: Eight Muturu cattle with an average weight of 270 ± 10.2kg were randomly selected from the cattle herd in the Teaching and Research Farm and used in the study. The animals were housed in four pens with two cattle placed in each pen. The experimental diets were fed to the animals *ad libitum* for twelve days after which 2.5kg of freshly voided excreta were collected in the evening of the twelfth day and subjected to anaerobic digestion in 10 litres bio-digesters. The biodigester contained 6kg of water and 2.5kg of freshly excreted manure from different treatments while the remaining space was left for gas accumulation.

Anaerobic Digestion of Excreta: The digestion lasted for 90 days. Total gas volume was collected through downward water displacement in gasometer after 24 hours. The volume of water displaced therefore equals the volume of gas produced. Sperian gas analyser (5N, 66424 USA) recorded the percentage of methane in the total gas and that percentage was used to calculate the volume of methane from total gas volume.

Substrate slurry was collected biweekly for pH, microbial population and volatile fatty acid analysis. The slurry pH was measured with a pH meter. One 1ml of each sample was serially transferred into nine 9ml of the sterile diluent (peptone water) with a sterile pipette and shaken vigorously. Serial dilution was continued until 10^6 dilution was obtained.

Aliquot portion (0.1ml) of the 10^6 dilutions was inoculated onto freshly prepared, surface-dried nutrient agar (NA). The inoculi were spread with a sterile (hockey stick-like) glass spreader to obtain even distribution of isolates after incubation. Nutrient agar and MacConkey agar plates were incubated for 24-48h at 37^0C. Anaerogen was used during the incubation process.

Enumeration of Microbial Population: Total plate counts for the nutrient and MacConkey Agar were done by counting colonies at the reverse side of the culture plates. Total colony count was expressed in colony forming units per millilitre (cfu/ml) (Harrigan and McCance, 1990).

Volatile fatty acid (acetic, propionic and butyric) concentration were determined using diluted volumes of 0.1ml in 10mls samples using Spectrophotometer (BB Bran England Model 7804C).

Ambient temperature during the experimental period was measured using thermometer.

Proximate and Statistical Analysis: The proximate composition of the experimental diets was carried out using AOAC. (2000). Data collected were analyzed using SAS (2002) while statistically different means were separated using Duncan's New Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Proximate composition of experimental diets.: Table 1 shows the proximate composition of the experimental diets. Protein values of experimental diets ranged from 9.49 to 9.63% which seemed adequate to support the anaerobic bacteria (Matt and Jeffrey, 2013). The percentage of fat in the experimental diets also ranged from 4.03 to 6.35. The components of the diet fed, especially type of carbohydrate, are important for methane production as they are able to influence the ruminal pH and subsequently alter the microbiota present (Johnson and Johnson, 1995).

Table 2 shows the digestibility (%) of the nutrients fed to the experimental animals. Results showed that crude protein, oil and crude fibre digestibility were significantly ($p<0.05$) affected by the addition of palm oil sludge. Digestibility of crude protein in T1 and T4 was significantly lower ($p<0.05$) than the other treatments with the least digestibility observed in T4. This suggests that the level of palm oil sludge inclusion that can affect protein digestibility is 30%. The digestibility of fat was also significantly different ($p<0.05$) among the treatments with treatment 3 having the least oil digestibility. The trend of crude fibre digestibility was slightly different. Crude fibre digestibility in treatment 1 and 4 were significantly lower ($p<0.05$) than crude fibre digestibility of T2 and T3. These results imply that crude fibre digestibility could be impaired if the level of palm oil sludge exceeds 20% in the diet. Pamquist (1994) indicated that fibre digestion will be restricted when ruminants receive diets with a fat content higher than 70g/kg dry matter intake.

Table 3 shows the gas production results during the experiment. There were significant ($p<0.05$) differences in total gas volumes produced from the different dietary treatments. Total gas volume increased from treatment 1 to treatment 3 and significantly declined in treatment 4. This suggests that percentage palm oil sludge in T4 might have suppressed the activities of microbes and their population in that treatment.

The volume of methane produced was also significantly ($p<0.05$) influenced by the treatments. Methane volume reached its peak in treatment 3 with 20% palm oil sludge implying that the optimum level of palm oil sludge in diet that enhanced methane production was 20% beyond which methane volume will be suppressed. Suppression effect of palm oil sludge may have resulted to the lowest methane volume recorded in treatment 4. This agrees with observations of Castillejos *et al*. (2007) who noted that a range of plant essential oils including garlic oil, cinnamon oil, eugenol, capsaicin, anise oil have all demonstrated beneficial effects on rumen fermentation that may improve productivity or reduce methane production. However, these results need to be confirmed *in vivo* under commercial production conditions. The oil may have either suppressed the activities of the microbial population or killed some protozoa that are co-host to methanogenic bacteria. The microbial population in the treatments were also significantly ($p<0.05$) different. The highest microbial population was recorded in treatment 1 having 0% palm oil sludge while the least microbial population was recorded in treatment 4 having 30% palm oil sludge. This suggests that the absence of palm oil sludge in T1 was responsible for the highest microbial population in that treatment since their activities were not suppressed. The least microbial population in treatment 4 with 30% palm oil sludge may be as a result of high level of oil in that treatment which suppressed the activities of microbial population. Calsamiglia *et al*. (2007) had earlier noted that supplementation of ruminant diets with essential oils can alter microbial populations, digestion and fermentation of diets, proteolysis, and methanogenesis on the rumen. Microbial populations can be altered when oil inhibits their growth and development.

There were significant ($p<0.05$) differences in the quantity of volatile fatty acids produced among the treatments. Treatment 3 which recorded the highest volume of gas and methane volume had highest levels of acetic acid. This is in line with the earlier assertion by De groot *et. al*. (1998) that methane is produced mainly when the feed is fermented to acetic and butyric acid in contrast to lower methane volume being produced with higher propionic acid production. Van Soest (1994) had earlier observed that low gas production is associated with propionic acid production. Getachew *et al.* (1998) had also noted that a shift in the proportion of VFA will be reflected by changes in gas production. According to Rahman *et al*. (2013), if fermentation of feeds leads to a higher proportion of acetic acid, there will be a concomitant increase in gas production when compared with a feed with a higher proportion of propionic acid. Propionic acid concentration was lowest as shown in table 3. This may be as a result of its conversion to acetic acid during digestion. Consequently, the limiting step in anaerobic digestion is hydrolysis, which is usually inhibited by high propionic concentrations (Juanga, 2005). The results of the experiment show that acetic acid concentrations decreased as the percentage palm oil sludge in diet increased while propionic acid concentration increased as the palm oil sludge concentration in the diet increased.

There were significant differences ($p<0.05$) in digester pH among treatments. The pH value of treatment 1 was significantly lower ($p< 0.05$) than those of treatments 2, 3 and 4. Molinuevo *et. al*. (2010) described the pH of anaerobic digestion to be near neutral pH but stressed that it depends on the loading rate and buffering capacity of substrate. The buffering capacity of animal waste such as cow, swine and poultry manure is high and that might be the reason of having pH values within a narrow range of 8.06 to 8.31.

Ambient Temperature during Digestion: The ambient temperatures recorded during anaerobic digestion are shown in Figure 1. The ambient temperature range during anaerobic digestion was between 27ºC-32ºC. The temperature on the first day of digestion was 29ºC but it came down to 27ºC on the second day and continued to fluctuate between a narrow range of 28ºC- 32ºC until the experiment ended. Choorit and Wisarnwan (2007) have described the temperature of anaerobic microorganism to be categorized into psychrophiles (<20ºC), mesophiles (25-37ºC) and thermophiles (55-65ºC). Barber and Ferry (2001) also described some methanogenic species that exhibit a preference of extreme heat of between 90-100ºC to be hyperthermophilic. Saleh and Mahmood (2004) noted that each microorganism has a certain temperature range within which it can grow and multiply and when temperature is increased within a certain range, chemical and enzymatic reactions increase at a faster rate and growth increases. Day 53 with highest temperature of 32ºC also coincided with the day of highest biogas production in treatment 1 which had no inclusion of palm oil sludge.

Biogas Production: Biogas production during the digestion process can be used to assess the interaction of microbial population responsible for digestion and subsequent production of gas and the inhibitory effect of palm oil sludge. Graphical representation of daily methane productions from different treatments are shown in figure 2 below.

Average daily methane yield observed from treatments (T_1, T_2, T_3, and T_4) were 288.76 litres/kg Dm, 250.78 litres/kg Dm, 321.65 litres/kg-Dm and 154.36 litres/kg Dm respectively. Treatment 3 produced the highest methane volume while T_4 produced the least average methane volume during the 90 days digestion process. The high volumes of methane observed in T_1, T_2, and T_3 imply that the suppressing effect of palm oil sludge on the methanogens was less on T_2, and T_3 than on T_4. This can also be attributed to the slurry pH and the proportion of volatile fatty acid production which can either favour methane production if more acetic and butyric acid are produced or reduce its production when more of propionic acid was formed.

Figure 2 shows that the highest peak of methane production occurred in T_3 followed by T_1 and T_2 while T_4 had the lowest peak of methane production. This could also be attributed to the suppressing effect of palm oil sludge in these diets on methanogens.

Total gas volume produced during the digestion process shows the relative proportion of methanogens to the total anaerobes within the anaerobic chamber. Peak total gas volume was also highest in T_3, T_2 and T_1 while T_4 had the lowest peak total gas volume. This suggests that the suppressing effect of palm oil sludge did not only affect the methanogens but all anaerobic bacteria within the digesters.

Figure 4 shows the proportion of volatile fatty acid produced during the fermentation process. There were higher levels of acetic and butyric acid production at the beginning and toward the end of digestion process. However, there were a significant reduction in acetic and butyric acid production between the 5th and 7th week of the digestion process probably due to the reduced activities of the methanogens. There was also higher production of propionic acid during this period and methane volume was equally low. A shift in the proportion of VFA generally reflects changes in gas production (Getachew *et al.*, 1998). Thus, lowest volumes of methane were produced at highest level of propionic acid production.

Table 1. Proximate composition of the experimental diets.

Treatment	$T_{1(0\%\ POS)}$	$T_{2(10\%\ POS)}$	$T_{3(20\%\ POS)}$	$T_{4(30\%\ POS)}$
Crude Protein(%)	9.63	9.61	9.49	9.49
Fat (%)	4.03	5.20	5.90	6.35
Crude Fibre (%)	5.77	5.82	6.48	5.88
Ash (%)	8.73	9.13	8.98	9.47

Table 2. Nutrient digestibility (%) of the experimental diets.

Treatments	T1(0% POS)	T2(10% POS)	T3(20% POS)	T4(30% POS)
Digestibility(%)				
Crude protein	**63.77^c**	**70.77^a**	**68.36^b**	**56.55^d**
Fat	**68.31^a**	**59.33^b**	**46.25^c**	**58.58^b**
Crude fibre	**68.40^a**	**64.93^b**	**68.04^a**	**62.57^b**

Means within the same row having different letters are significantly different (P <0.05).

Table 3. Gas production values during the anaerobic digestion of the cattle excreta.

	Treatments			
Parameters	T_1	T_2	T_3	T_4
Total gas volume(Ml)	*54255.00^a*	*55342.00^a*	*61823.50^a*	*30385.00^b*
Methane volume(Ml)	*23227.55^b*	*20563.55^b*	*26375.40^a*	*12675.33^c*
Total acetic acid(mg/l)	*26.544^a*	*20.59^b*	*24.12^{ab}*	*20.57^b*
Total butyric acid (mg/l)	*26.18^b*	*20.86^b*	*22.30^b*	*32.97^a*
Total propionic acid(mg/l)	*15.47^c*	*19.53^{ab}*	*18.27^b*	*21.10^a*
Total microbes(cfu/ml)	*$3.36*10^{9a}$*	*$2.64*10^{9b}$*	*$2.17*10^{9c}$*	*$2.11*10^{9c}$*
pH of digester	*8.06^b*	*8.24^a*	*8.31^a*	*8.24^a*

Means within the same row having different letters are significantly different (P <0.05).

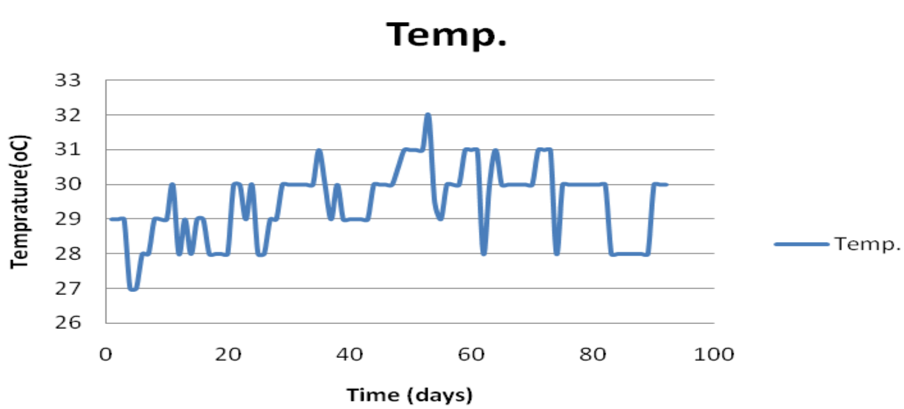

Figure 1. Ambient temperature during anaerobic digestion of manure.

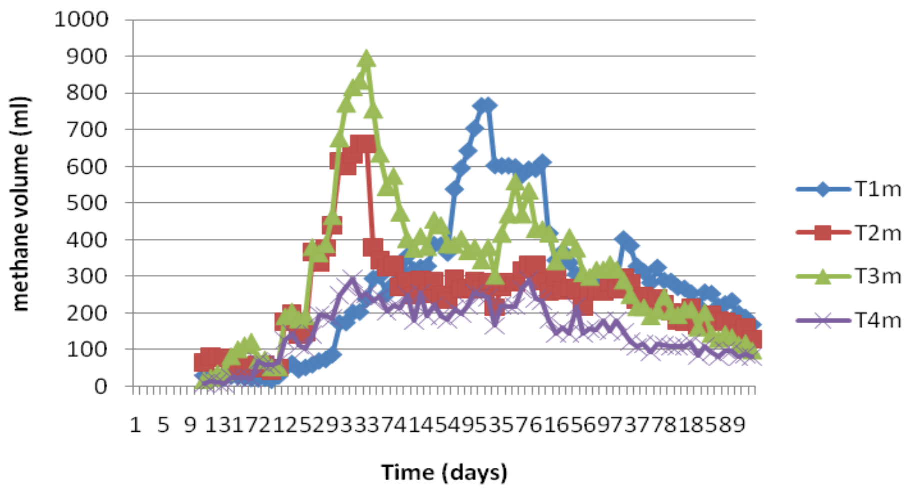

Figure 2: Daily methane production during the digestion period

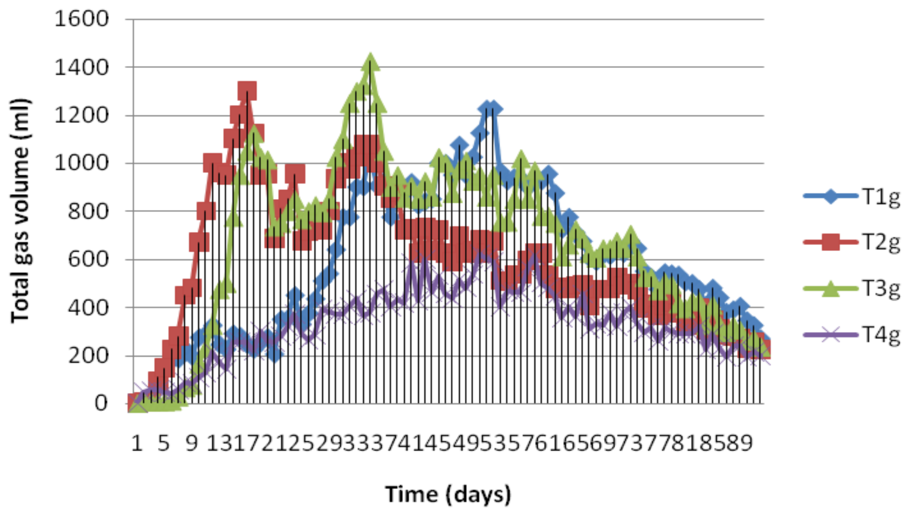

Figure 3: Total gas volume produced during the digestion process.

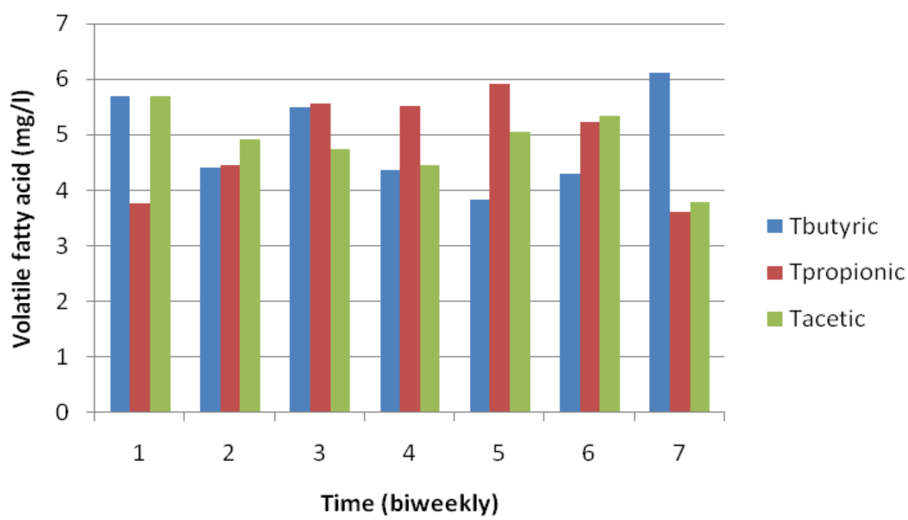

Figure 4: Proportion of volatile fatty acids (acetic, propionic and butyric) production during the digestion process.

Conclusion: The results of the experiment showed that addition of palm oil sludge in cattle diets affected the digestibility of experimental nutrients, the pH and methane production from the manure. Microbial population during anaerobic digestion of manure also decreased as the palm oil sludge increased. Ambient temperature of 32ºC on 53rd day of the fermentation process also recorded the highest gas production from the manure. Volatile fatty acids (acetic, propionic and butyric) production were also affected. However, enhanced acetic and butyric acid production with a concomitant propionic acid reduction increased methane production.

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