METHANOGENESIS IN WEANER PIGS FED DIFFERENT FIBRE SOURCES USING SWINE FAECAL MATTER AS COMPARED WITH RUMEN LIQUOR

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Abstract
The study was carried out to compare the methane production between filtrate from swine's faecal slurry and the use of rumen liquor from goat. The swine for this study was fed for twenty-one days with different dietary treatments. Faecal samples were collected from the pigs and processed for pre and post *in vitro* parameters. Rumen liquor (RL)+treatment (Trt) one (RL+Trt1) had the overall highest methane production at the 24th hour while the overall least methane production at the 24th hour was seen in Swine faecal (SF)+ treatment 3 (SF+Trt3). For post *in vitro* parameters, the highest methane (CH_4) production was 21% which was associated with RL+Trt1 and RL+Trt2. The dry matter digestibility (DMD) was highest in the control having (81.87%). Methane gas volume (CH_4-GV) produced was highest in SF+Trt3 with a value of 0.93%. The same trend was observed for Fermentation Efficiency (FE) and Methane Reduction (CH_4RED) with highest FE value of 17.64 and CH4RED of 97.44%. The trend was however different for Short Chain Fatty Acid (SCFA) as RL+Trt1 had the highest value of 0.84%. In the light of the above, when other beneficial factors are placed alongside potential methane production, Treatment 3 with 20% Rice bran is advocated for use by farmers as it had a significant reduction in faecal methane being released into the environment.

Keywords: Methanogenesis, Fibre sources, Rumen liquor, Faecal Matter and Weaner pigs

1. INTRODUCTION

Pork is the most widely consumed meat product in the world, and pig production is the second highest contributor of Green House Gas (GHG) emissions from the livestock sector (FAO, 2011). According to Mi *et al*. (2019) methane emissions from pigs account for 10% of total methane production from livestock in China. They also emphasized that methane emissions do not only contribute to global warming, as it has 25 times the global warming potential (GWP) of CO_2 and also represent approximately 0.1~3.3% of digestive energy loss. It has been predicted that by the year 2050, worldwide pig consumption will increase by almost 40% (FAO, 2011). Subsequently the need to expedite action in providing better feed management plan to forestall the negative consequences associated with the rise in production becomes necessary. Methane is regarded as the second most significant anthropogenic greenhouse gas, and its global warming potential is more than 25 fold higher than that of carbon dioxide (CO_2) (Thauer *et al.*, 2010). Livestock lose a significant amount of digestible energy to methane gas production which is a threat to the environment and production loss to the farmer. For instance, the intake of 300g of dRes (digestible residue) is accompanied with the enteric production of 36g CH_4 by fattening pigs and 6.3g CH_4 in matured sows. Also, enteric emissions accounts for energy losses of 56.65 kJ per g of CH_4 produced, which represents about 0.4–0.5% of digestible energy (DE) for fattening pigs and 1.0–1.5% DE for adult sows (Philippe and Nicks, 2014). Some studies on different grasses and browse plants used in livestock production in Nigeria have been carried out. Ahamefule *et al*. (2006) assessed the nutritive value of some plants browsed by cattle in Umudike, Southern Nigeria. Ajayi and Babayemi (2008) also worked on the comparative in vitro evaluation of mixtures of *Panicum maximum*, Ntchisi with Stylo (*Stylosanthes guianensis*) Lablab (*Lalab purpureus*) Centro (*Centrsema pubescens*) and Histrix (*Aeschynomene histrix*). Akinfemi *et al*. (2009) have also worked on the use of an *in vitro* gas technique to evaluate some Nigerian feed stuff. However, there is scarcity of information on methanogenesis in pigs and effect of plant sources on methanogenesis in pigs. This study was therefore designed to identify which of the fibrous feedstuffs emits the most methane in pig faecal samples, and to recommend to farmers, fibrous feed materials useful in pig farming.

2. MATERIALS AND METHODS

The experiment was conducted at the Piggery Unit of the Department of Animal Science, Faculty of Agriculture, University of Port Harcourt, Choba. A total of twelve (12) weaner

pigs (6 males and 6 females) were used for the experiment. They were housed in the piggery facility for a period of twenty-one (21) days, and all daily routine management practices were carried out. No medication was administered within the period of the experiment. A completely randomized design was used for the experiment. The twelve (12) weaner pigs (6 males and 6 females) were divided into three (3) treatments with two (2) Replicates and two (2) animals per replicate. The fibrous feedstuffs tested were; cassava peels, wheat bran and rice bran. They were used to formulate an isonitrogenous and isocaloric diets and fed to the pigs on daily basis. Treatment one (T1) had 20% Cassava peels; Treatment two (T2) had 20% Wheat bran, and Treatment three (T3) had 20% Rice bran. Data on body weight, feed consumption were collected on a weekly and daily basis respectively. Blood was collected into Ethylene diamine tetra acetic acid (EDTA) bottle from the jugular vein of each pig and stored in an ice pack while in transit to the laboratory for the determination of hematological parameters. Determination of the Dry matter (DM), Organic matter and Ash were done using the procedure as described by the Association of Analytical Chemist (AOAC), (1990) while Neutral detergent fibre, Acid-detergent fibre and lignin was determined using Van Soest *et al.*, (1991). Proximate analysis of the formulated experimental diets was conducted to determine the energy, crude protein and crude fibre contents of the diets.

Drying of faecal samples can result in the loss of some chemical components, and underestimation of the Kjeldahl nitrogen values, may occur as a result of ammonia loss during drying of faeces; consequently, the faecal samples were stored in a fridge to prevent ammonia loss (Ministry of Agriculture, Fisheries and Food, 1981). Other processes were ascertained following the laid down procedures described by AOAC, (1990). Faecal samples were collected from the fridge and mixed with distilled water to form slurry which was later filtered using a two layer cheese cloth and the liquid was collected into a beaker. Buffers solution was added to the filtered slurry and the mixture was later transferred into a beaker kept in a water bath of 39°C which is to simulate the stomach temperature. The mixture of filtered faecal slurry and buffers was continuously flushed with CO_2 to maintain an anaerobic condition. 30ml inoculum was collected into a 100 ml calibrated syringe containing the bagged samples and latter arranged in the incubator at 39°C. Syringes for the experiment were numbered. ANKOM bags measuring 3cm in breath and 7cm in length were sequentially numbered to tally with the numbering on the syringes. The edges were trimmed and the bags were weighed and recorded. 200mg of each treatment diet sample was weighed into the bags and sealed using nylon sealing machine at 2-5°C which were latter placed in the syringe

containing the substrate. After the recording of gas volume, the ANKOM bags were removed washed and oven dried at 70-80°C for 24 hours before taking the final reading of sample left in the bag. Rumen inoculum in the present study was collected using suction tube from six (6) goats (sex not considered) i.e. the suction tube was passed through the mouth into the rumen of the goats (*Capra aegagrus hircus*). *Panicum maximum* 30%, *Centrosema mole* 30% and concentrate (growers mash) 40% were the constituents of the feed fed the donor animals for a period of four days prior to collection of inoculums. Rumen liquor from the donor animals were collected into a warm two (2) litter flask whose aim is to prevent the microbes from cold shock while in transit from the farm to the laboratory. On arrival at the lab, buffers solution was added to the filtered rumen liquor which was later transferred into a beaker kept in a water bath at 39°C which was to simulate the rumen temperature and prevent cold shock. 30ml inoculum was collected into a 100 ml calibrated syringe containing the bagged samples and latter arranged in the incubator at 39°C.

Gas production (GP) profiles were determined manually using the syringe method. *In-vitro* fermentation system was set up following the procedure as described by the International Livestock Research Institute (Ogbai and Berhan, 1997). 200mg of each faecal sample was weighed into ANKOM bags which were subsequently placed in a 100 ml syringe containing the rumen liquor with a silicon tube tightly a fixed to the end of the syringe and a clip to prevent gaseous escape. The syringes were placed in an incubator at 39°C. The volume of gas in the piston was read three (3) hourly after the start of incubation. The final reading was taken at 24th hour. A blank determination was done to determine gas production (GPo) from only the inoculum at each reading. During the incubation period, the plunger moves upward signifying gas production at each interval. The gas produced is due to anaerobic fermentation. At the end of the 24 hours incubation period, 2ml of NaOH with a concentration of 40%, was infused into each of the incubated syringe via the silicon tube. The syringe was shaken vigorously and an observable inward movement of the syringe plunger was noticed, which signifies CO_2 removal. The difference in volume of gas produced before the injection of 2ml of 40% NaOH and that observed after, is the quantity of methane gas produced.

Statistical Analysis

All data including rumen fermentation, Dry matter content (DM), Crude protein (CP), were sorted using Microsoft Excel. Data were subjected to Analysis of Variance (ANOVA) using SAS (2004) with significant means separated with DMRT.

Table 1: Composition of the Experimental Diets

INGREDIENT	TREATMENT 1 (%)	TREATMENT 2 (%)	TREATMENT 3 (%)
Maize	37.5	41	40
Palm Kernel Cake	13	10.5	12
Groundnut cake	6.5	6	7
Blood meal	11	8.5	8.5
Wheat bran	0	20	0
Cassava peels	20	0	0
Rice bran	0	0	20
Groundnut oil	4	6	4.5
Bone meal	3	3	3
D-L Methionine	0.1	0.1	0.1
Lysine	0.1	0.1	0.1
Premix	2.5	2.5	2.5
Salt	2.3	2.3	2.3
Total	100	100	100
Calculated nutrients			
ME: Kcal/kg	2,817	2,806	2,818
Crude Protein (%)	18.26	18.02	18.14
Crude Fibre	5.76	5.34	5.49
Analyzed nutrients			
ME:Kcal/g	2,800	2,798	2,810
Crude Protein (%)	18.10	18.00	18.01
Crude Fibre (%)	5.64	5.23	5.50

3. RESULT

Significant gas production started at the 6th hour. Rumen Liquor (RL) + Treatment (Trt) 2 (RL+Trt2) had the highest gas production of 15.00ml at the 6th hour, although not significantly different (P>0.05) from the control but was significantly different (P<0.05) from the blank, SF+Trt1, SF+Trt2 and SF+Trt3 which were not significantly different (P>0.05) from each other. A similar trend was observed from the 9th hour of incubation to the 24th hour, where Rumen Liquor (RL) + Treatment (Trt) 2 (RL+Trt2) showed higher significant (P<0.05) values from the other treatments except the control.

RL+Trt1 and RL+Trt2 had the highest Methane (CH_4) production percentage of 21.50%, but it was not significantly different (P>0.05) from the other treatments, except SF+Trt1 and SF+Trt3. The Dry Matter Digestibility (DMD) did not show any significant difference (P>0.05) amongst the various treatments, while the Methane Gas Volume (CH_4GV) results revealed that SF+Trt3 had the highest value and was significantly different (P<0.05) from the other treatments except the blank. Fermentation Efficiency (FE), also showed a similar trend. SF+Trt3 had the highest value fermentation efficiency and was significantly different (P<0.05) from all other treatments. Methane Reduction (CH_4red) showed that SF+Trt1 and SF+Trt3 had the highest percentages and were significantly different (P<0.05) from all other treatments except RL+Trt3, SF+Trt2 and the blank. SF+Trt3 had the least Short Chain Fatty Acid (SCFA), although it was not significantly different (P>0.05) from SF+Trt1, but was significantly different (P<0.05) from all other treatments, as RL+Trt1 had the highest value.

Table 2: Gas Production at Different Incubation Hours for Rumen Liquor/Swine Faecal Sample

Treatment combinations	3 Hours	6 Hours	9 Hours	12 Hours	15 Hours	18 Hours	21 Hours	24 Hours
Rumen Liquor + Treatment 1	8.50^{a}	14.50^{ab}	20.00^{a}	25.50^{a}	27.50^{a}	30.50^{a}	38.00^{a}	38.00^{a}
Rumen Liquor + Treatment 2	9.00^{a}	15.00^{a}	19.50^{a}	23.00^{ab}	28.00^{a}	29.50^{ab}	34.50^{ab}	35.00^{a}
Rumen Liquor + Treatment 3	5.00^{b}	10.50^{bc}	14.00^{ab}	17.00^{bc}	18.50^{bc}	21.50^{bc}	24.50^{bc}	26.00^{ab}
Swine Faecal + Treatment 1	0.00^{c}	0.50^{d}	1.50^{c}	2.00^{d}	4.50^{d}	8.00^{d}	9.50^{de}	10.00^{b}
Swine Faecal +Treatment 2	0.00^{c}	2.00^{d}	3.00^{c}	5.00^{d}	8.00^{d}	11.00^{d}	16.00^{cd}	25.00^{ab}
Swine Faecal +Treatment 3	0.00^{c}	1.50^{d}	1.50^{c}	2.00^{d}	2.50^{d}	3.00^{d}	4.00^{e}	5.00^{c}
Control	6.44^{ab}	12.67^{abc}	17.56^{ab}	21.56^{ab}	25.56^{ab}	28.89^{ab}	31.11^{ab}	34.22^{a}
Blank	6.67$_{ab}$	8.67c	12.00^{b}	13.33^{c}	16.67^{c}	19.33^{c}	24.67^{bc}	24.67^{ab}
Standard error of mean	0.652	1.021	1.382	1.683	1.813	1.885	2.168	2.485

a, b, c, d, e: Means on the same column with same letter are not significantly different (P>0.05).

Table 3: Post *In-vitro* Parameters for Rumen Liquor/Swine Faecal Sample Used for the Experiment

Treatment combinations	Methane ml	Dry Matter Digestibility g/100g	Methane Gas Volume%	Fermentation Efficiency	Methane Reduction%	Short Chain Fatty Acid/200mgDM
Rumen Liquor +Treatment 1	21.50[a]	72.75	0.56[c]	1.91[b]	87.78[b]	0.84[a]
Rumen Liquor Treatment 2	21.50[a]	69.20	0.61[bc]	2.01[b]	87.78[b]	0.77[a]
Rumen Liquor +Treatment 3	15.50[ab]	59.50	0.58[bc]	2.99[b]	91.19[ab]	0.56[ab]
Swine Feacal +Treatment1	4.50[b]	62.67	0.45[c]	6.50[b]	97.44[a]	0.17[bc]
Swine Feacal + Treatment 2	15.50[ab]	61.17	0.51[bc]	4.38[b]	91.19[ab]	0.53[ab]
Swine Feacal +Treatment 3	4.50[b]	63.22	0.93[a]	17.64[a]	97.44[a]	0.05[c]
Control	19.55[a]	81.87	0.58[bc]	2.55[b]	88.88[b]	0.75[a]
Blank	17.33[ab]	67.40	0.74[ab]	2.38[b]	90.15[ab]	0.52[ab]
Standard error of mean	1.623	3.303	0.032	1.068	0.922	0.059

a, b, c: Means on the same column with same letter are not significantly different (P>0.05).

4. DISCUSSION

Gas production varied significantly from the 6th to the 24th hours of incubation. The 3rd to 12th hour of gas production ranged from 0 - 25.50ml. The variation in the volume of gas produced could be attributed to the varying sources of the fibre in the diet of the pigs. These values are higher than those reported by Nguyen *et al.* (1997). They reported the highest gas production value of 0.72 ml at 24th hour against the 38.00ml observed in this study. The results generally showed that the rumen liquor produced more gas than the swine faecal matter, which is an indication that ruminant animals produce more GHG than non-ruminants. This is in support of Jensen (1996) who observed that methane production by monogastric animals is lower than methane production by ruminants. The non significant results shown amongst the three Swine faecal matters obtained from different fibre source could indicate that fibre sources in the diet did not influence the production of gases. Methane (CH_4) produced across treatments was lower than that reported of Baraka and Abdi-Rahman, (2012). This variation could be attributed to the population of methanogenic bacteria present in the distal colon and caecum of the pigs. Jensen (1996) in his work on methanogenesis in monogastric animals observed that in pigs, the population of methanogenic bacteria is more than 30 times as dense in the distal colon as in the caecum explaining that the rate of methane production is much higher in the colon than in the ceacum. These methanogens play an important role in maintaining the balance of the gut microbiome and in pigs, the large intestines are the main habitat for the microbiome. According to Mi *et al*. (2019) in their work on diversity and community of methanogens in the large intestine of finishing pigs, they observed that the major methanogen in the large intestine of finishing pigs was Methanobrevibacter and went further to suggest that the seventh order Methanomassiliicoccales and species Methanosphaera stadtmanae present in the large intestine of pigs might contribute to the transfer of hydrogen and fewer methane emissions. It has also been observed that the amount of methane excreted clearly seems to depend on the amount of non-starch polysaccharide intake and that the directly measured methane production rate in pigs is from 3.3 to 3.8 times lower than the amount expected from stoichiometric estimates (Baraka and Abdi-Rahman, 2012).

The dry matter digestibility (DMD) of the test diets had no significant difference. This could mean they have similar fibre content. However, the values were above those reported by Kilic and Garipoglu, (2009) in their work on *in situ* rumen degradability, *in vitro* digestibility and *in vitro* gas production of full fat canola seeds. Methane gave volume (CH_4-GV) significantly varies across treatment combinations. This could be as a result of the composition of the diets and well as the volume of the microbiome present in the pigs. The values reported in this this study were below those reported by Baraka and Abdi-Rahman, (2012) in their work on *in vitro* evaluation of sheep rumen fermentation pattern after adding different levels of eugenol- fumaric acid combinations. Fermentation efficiency (FE) also varied significantly across the treatment combination. This corresponds with the significant varying methane gas produced. The values for fermentation efficiency for this study were however lower than those reported by Baraka and Abdi-Rahman, (2012). Also, Bamikole *et al*. (2019) in their preliminary *In vitro* Screening of some spices and medicinal plants from Edo and Rivers States, Nigeria for reducing enteric methane production in ruminants reported a range that was below those obtained in this work. The values for short chain fatty acid (SCFA) reported in this study significantly varied across treatment combinations but were below that obtained by Nguyen *et al.,* (1997) in their work on *In vitro* gas production and washing losses of tropical crop residue for ruminant and pigs. From this study, it can be concluded that rice bran inclusion in the diets of pig is good as it has reasonably increased fermentation efficiency which eventually leads to low levels of Short Chain Fatty Acid production in swine faecal samples. It is therefore suggested that Treatment 3 with 20% Rice bran be advocated for to farmers as it had a significant reduction in faecal methane being released into the environment.

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