

## **Effect of Co-Digestion of Cow Dung And Poultry Manure on Biogas Yield, Proximate And Amino Acid Contents of Their Effluents**

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**Abstract:** A study was carried out on the effects of co-digestion of cow dung and poultry manure on biogas yields, proximate and amino acid compositions of the by-products. Five mixed substrates: 100:0 (cow dung :poultry manure), 0:100 (cow dung :poultry manure), 50:50 (cow dung :poultry manure), 75:25 (cow dung :poultry manure) and 25:75 (cow dung :poultry manure), represented treatments A, B, C, D and E respectively. These in triplicates, were separately loaded into 13.6L digesters locally fabricated and kept for an eight week retention period. The average biogas yields obtained ranged from 2961.0 - 2031.1ml, in the order of 50:50 (cow dung : poultry manure) > 25:75 (cow dung : poultry manure) > 0:100 (cow dung : poultry manure) > (cow dung : poultry manure) > 75:25 (cow dung : poultry manure). Anaerobic digestion of the wastes led to enhancements of %ash and moisture content, while % reduction in the total lipid, total solid and volatile solid contents ranged from 40.38- 86.82%, 11.77 - 24.82%, and 39.99-59.00%, respectively. The crude protein (CP) content increased for all treatments except B, with 5.74% as % reduction. Similarly, only treatments B and E had 18.05% and 32.11% respectively as % reductions in nitrogen free extract (NFE) after digestion. The mixed digested substrates recorded remarkable reductions in % ash, NFE, TS and VS while there were increases in the CP and MC than the single substrates. The histidine, glycine, cysteine and methione contents were noticeably enhanced for all the by-products. The leusine, threonine and isoleusine contents increased for all digested treatments except B, with 60.14, 6.14 and 16.89% as percentage reductions respectively. Digested 100:0 cow dung had the highest % increase for threonine, proline, glycine, alanine and total amino acid. Among the co-digested substrate, 50:50 ratio recorded the highest % increase for glutamic acid, glycine, alanine and methione; the 75:25 ratio for histidine, serine, isoleusine, leusine total amino acid, total essential amino acid, total non-essential amino acid and total sulphur amino acid, while the 25:75 ratio was for threonine, proline, tyrosine and total aromatic amino acids due to anaerobic digestion.

**Keywords:** Biogas; Co-Digestion; Cow Dung; Poultry Manure; Proximate; Amino Acid; Effluents

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### **I. Introduction**

In the last few decades, livestock waste generations have increased with the demand for livestock products. Inadequate management strategies and indiscriminate disposal of these agricultural and municipal wastes often pose socio-economic, environmental and health risk, as they constitute ugly scenes, generate nauseating odors, breeding ground for pathogenic microbes rodent and flies (1,2, 3), as well as sources of water pollution, ammonia and greenhouse gases (GHGs) emissions, P and heavy metal contaminations of soil and water.

Biodegradation of agricultural wastes is a biomass conversion technology (4, 5) that produces useful gas from organic matter through the process of anaerobic digestion. Verma (6) pointed out that anaerobic digestion is one technology that can successfully treat organic fraction of wastes, which involves three stages: hydrolysis, acidogenesis and methanogenesis (7, 8). It has the advantage of producing energy (biogas), high quality bio-fertilizer, reduce C-emission while preventing the transmission of pathogenic organisms (1, 9). Biodegradation of livestock wastes can also be used as manure in pond fertilization, in fish farming (10).

Co-digestion of manure and organic wastes has proven to be a very successful way of improving biogas production. This is because manure with recorded low biogas yield, when codigested with easily degradable organic waste, boost their production. Co-digestion helps to concentrate wastes, as high water content in manure dilutes concentrated organic waste which could be inhibitory and difficult to treat separately (11).

El-Deeket *et al.*, (12), pointed out that composting, anaerobic digestion, combustion, oxidation and drying are possible ways of enriching livestock manure and organic wastes as useful protein sources. The technology has been reported to increase protein and total protein, albumin, globulin, aspartate amino transaminase, alanine

amino transaminase as well as improved content and availability of essential amino acids in the feed (13). El-Deek *et al.*, (12), reported increase in crude protein and essential amino acid contents of fermented dried poultry manure (DPM) except for glycine, histidine and tyrosine.

Various cultures of microorganisms can be used to improve utilization and conversion of the manure nitrogenous materials to protein. Vuori and Nasi (14), used microbial strains for the efficient elimination of uric acid by fermenting poultry manure. According to El-Deek *et al.* (12), fermentation of DPM, using *Candida utilis*, increased the crude protein content from 19.1 to 24.9%, while decreasing the non-protein nitrogen (NPN) and uric acid. Also, the amino acid content of fermented DPM were greatly increased than those of the DPM except for glycine, histidine and tyrosine. Present study therefore focuses on the effects of co-digestion of cow dung and poultry manure on biogas yields and some biochemical constituents of the by-products.

## II. Materials and Methods

### 2.1 Substrate preparation

The test organic waste substrates (cow dung and poultry manure) were locally sourced from the animal units Federal College Forestry, Jos, Nigeria. Dried samples of these substrates were subjected to pre-anaerobic digestion treatments of homogenization (pulverization, using mortar and pestle were separately pulverized to achieve 2-4mm particle size (15), screening (involving sorting, separation and removal of fibrous solids and extraneous objects from each of the homogenized substrates, (16), mixing (the substrates were mixed in the following predetermined ratios (w/w) for both single and mixed substrates (co-digestion) as shown in Table 1, and storage (The substrates in their different ratios were packed in sterile black polythene bags (1), to conserve moisture and stored in a cool dry place below 20°C (16, 17).

**Table 1:** Treatment description

Treatment	Description	Ratio
A	Cow Dung	100:0
B	Poultry Manure	100:0
C	A + B	50:50
D	A + B	75:25
E	A + B	25:75

### 2.2 Anaerobic Digestion Study

#### 2.2.1 Slurry preparation, loading and biogas measurement

The slurry of sample of each of the treatments were made by separately mixing 1000g of each samples with 3000ml of sterile distilled water in a 1:3 ratio w/v, (1, 18, 19). The batch fermentation approach was adopted. The slurry of sample of treatment A was loaded into sterilized digesters (13.6L capacity), using sterilized metallic funnel through the central opening, which was immediately covered with a brass lid and firmly sealed to ensure air-tight (providing anaerobic condition), following the fitting of thermometer and gas delivery pipe, using rubber corks. The same procedure was followed for samples from treatments B to E. Triplicate of each set-up (making a total of fifteen (15) experimental units) were arranged in a completely randomized design (CRD) in an experimental cubicle, where uniform temperature was maintained. The digesters were manually shaken for one minute thrice daily to ensure homogenous substrate condition. The digestion lasted for 56 days (8 weeks) retention time (1, 20, 21). The biogas production (in dm<sup>3</sup>/kg) was measured by downward displacement of water by the gas (22), over an eight week period.

#### 2.2.2 Proximate analysis of substrates and spent slurry

Dried samples of raw and digested substrates (A to E) were separately pulverized using mortar and pestle. The samples were analyzed for parameters such as moisture content (MC), crude protein (CP), crude fibre (CF), total fat (TF), nitrogen free extract (NFE), total ash (TA), total solid (TS) and volatile solid (VS), total nitrogen (TN) and total organic carbon (TOC) according to the procedure of AOAC (23).

#### 2.2.3 Determination of Amino Acid Composition of Digested Samples

The amino acid composition of both raw and the digested samples were determined using AOAC (23) and Spackman's methods as described by Muhammad and Oloyede (24). The samples were dried to constant weight, defatted, hydrolyzed and evaporated using a rotatory evaporator before loading into a Technicon Sequential Multi-sample (TSM) amino acid analyzer. This involved defatting, hydrolysis and the use of Technicon Sequential Multi-sample (TSM) amino acid analyzer.

### **2.3 Statistical analyses**

The data obtained were subjected to analysis of variance (ANOVA) and the respective significant means were separated using the Duncan multiple range test, this was conducted using SPSS15.0 version(22)

## **III. Results And Discussion**

### **3.1 Results**

#### **3.1.1 Volume of Biogas Production during Anaerobic Digestion of Samples**

The results of average volume of biogas produced (ml) during the 8 weeks of digestion (WOD) of samples of all the treatments is shown on Tables 2.

All treatments had a general increase in the average volume of biogas production from the 1<sup>st</sup> to the 6<sup>th</sup> weeks of digestion (WOD), which gradually decreased from the 7<sup>th</sup> to the 8<sup>th</sup> WOD. Treatment C recorded the highest average volume (ml) of biogas (AVB) production throughout the eight WOD, with an average volume of 621.0ml which reduced to 429.7ml by the 8<sup>th</sup> week. Conversely, treatment D had the lowest AVB production from week 1 up to the 7<sup>th</sup> WOD, but at the 8<sup>th</sup> week, the 100% poultry manure (treatment B) gave the lowest AVB of 184.3ml. Analysis of variance (ANOVA) on weekly data indicated significant difference ( $p < 0.05$ ) in average volume of biogas produced throughout the period of digestion.

The average cumulative biogas production ranged from 2031.1ml - 2961.0ml, in the order of 50:50 (cow dung : poultry manure) > 25:75 (cow dung : poultry manure) > 0:100 (cow dung : poultry manure) > (cow dung : poultry manure) > 75:25 (cow dung : poultry manure) as presented in Figure 1. Apart from ratio 75:25, the mixed substrates ratios 50:50 and 25:75 had better cumulative mean biogas production than the single substrates.

#### **3.1.2 Proximate Composition of Samples before and after Anaerobic Digestion**

The results of the biochemical composition of all the treatments before and after anaerobic digestion are revealed on Table 3. There were increases in ash and moisture contents for all treatments, after anaerobic digestion (AD), with treatments A (55.93%) and C (530.43 %) and D(45.54%) and B(264.94%) recording the highest and lowest values of % increases for ash and moisture contents (MC) respectively. The Total lipid (TL), total solid (TS) and volatile solid (VS) contents decreased in all treatments after AD. Treatments A and C, and E and B had 86.82 and 24.82%, and 40.38% and 11.77% as the highest and lowest % decrease in TL and TS respectively. The volatile solid contents of the digested wastes ranged from 39.99-59.00%. Anaerobic digestion resulted in percentage increase in Crude protein (CP) contents for all treatments except B, with 5.74% as % reduction. Similarly, only treatments B and E had 18.05% and 32.11% respectively as % reductions in nitrogen free extract (NFE) due to anaerobic digestion. The mixing ratios of the organic substrates had varying influences on the anaerobic digestion of the wastes. The mixed digested substrates recorded remarkable reductions in % ash, NFE, TS and VS and increases in the CP and MC than the single substrates.

#### **3.2.3 Carbon –Nitrogen Ratio of Samples before and after Anaerobic Digestion**

The carbon – nitrogen (C/N) ratios of the substrates as presented on Table 4 ranged from 12.82 – 21.38, before anaerobic digestion (AD), with treatments C and D recording the highest and lowest values respectively. However, after AD, the C/N ratio ranged from 7.93 – 13.02, with treatment A having the highest. All substrates recorded had remarkable percentage reductions after AD. Treatment E and B had 59.27% and 12.94% as the highest and lowest % reduction of C/N after digestion. The mixed substrates recorded higher % reduction than the single substrates.

#### **3.2.4 Amino Acid Composition of Samples before and after Anaerobic Digestion**

The results of the amino acid composition (g/100g of protein) of samples of all the treatments before and after anaerobic digestion (AD) are shown on Table 5. The results showed variations in the amino acid profile for all treatments. Before AD, treatment B had the highest contents of arginine, threonine, glutamic acid, proline, alanine, valine, isoleucine, leucine, tyrosine and phenylalanine, total amino acid (TAA), total essential amino acid (TEAA), total non-essential amino acid (TNEAA) and total aromatic amino acid (TArAA). Treatment E was richest in histidine, serine, cysteine, methionine and total sulphur amino acid (TSAA); while treatments A, C and D had more lysine, aspartic acid and glycine respectively. After anaerobic digestion, all treatments had increase in the levels of histidine, glycine, cysteine and methionine, and decrease in phenylalanine, aspartic acid and valine. Leucine, isoleucine and threonine contents increased for all treatments except B, with 60.14%, 16.89% and 6.94% as percentage reductions for these amino acids respectively. However, only treatment D gave 9.89% as percentage increase while all other digested substrates recorded various percentage decreases of Lysine content after anaerobic digestion. Anaerobic digestion of the samples resulted in general increase in the total amino acid (TAA) and total essential amino acid (TEAA) contents of all the treatment samples except B with 7.59% and 24.44% as percentage reductions on these parameters

respectively. All the digested substrates had relative increase in total non-essential amino acids (TNEAA) and total sulphur amino acid (TSAA). 100% cow dung (treatment A) had 48.47% and 400.00% as %increases for TNEAA and TSAA, while 100% poultry manure (treatment B) and 25:75 cow dung + poultry manure (treatment E) with 10.47% and 147.4% as percentage decreases on both parameters respectively. All treatment samples recorded reduction in total aromatic amino acid (TArAA) content, except for treatments E(1.12%). Generally, the by-product of digested 75:25 mixed substrate gave the highest lysine, histidine, isoleucine, leucine and TEAA contents. The 25:75 ratio had the highest for tyrosine and TArAA. Digested 100:0 cow dung had the highest % increase for threonine, proline, glycine, alanine and TAA. Among the co-substrate ratios used, 50:50 ratio recorded the highest % increase for glutamic acid, glycine, alanine and methione; the 75:25 ratio was for histidine, serine, isoleucine, leucine TAA, TEAA, TNEAA and TSAA, while the 25:75 ratio threonine, proline, tyrosine and TArAA due to anaerobic digestion.

### **3.2 Discussion**

#### **3.2.1 Effects of Anaerobic Digestion of Samples on Biogas Yields**

The generally increase in biogas production with digestion time up to the 6<sup>th</sup> week of digestion agrees with Li *et al.*, (25), who adduced the initial increase in biogas production to the presence of readily biodegradable organic matter and high content of methanogens in all the substrates. Kaosol and Sohgrathok (26), related the stoichiometric conversion of methane production directly to organic degradation indicating that 1.0g of COD removal equals 395 mL methane (27). Before digestion, all substrates had higher values of total solids (TS), volatile solids (VS), % organic carbon %OC, and % chemical oxygen demand %COD, which became reduced at the end of the digestion (25). Jha *et al.* (28), reported close relationships between biogas yield and TS, VS, COD and TOC removal. El-Mashad and Zhang, (29), affirmed that biogas production increase with an increase in COD removal and VS reduction. They suggested that the methanogenic consortium acclimated very well and consequently leads to the digestion of organic matter (COD) and volatile solid (VS) under anaerobic condition. The reduction in volume of the biogas produced after an initial sharp increase, corroborated the findings of Xie *et al.* (30). This is due to lack of soluble biodegradable organic substances, accumulation of volatile fatty acids (VFAs) and a low pH. Proteins are also known to influence methane formation positively and therefore a high methane yield can be attained from substrates rich in proteins (31). Concerning the methane production rate constant, positive correlations were only found with P, Ca, Mg and K.

#### **3.2.2 Effects of Different Mixing Ratios of Substrates on Biogas Yields**

The highest cumulative average volume of biogas (CAVB) recorded for treatment C 50:50 (cow dung/poultry manure mixture) at the end of 8 weeks of digestion (WOD) agrees with findings of Lehtomaki *et al.* (32), who reported an optimal yield with mixing ratio 1:1 when cattle manure, grass silage, sugar beet tops and oat straw were co-digested. The biogas yield was significantly ( $p < 0.05$ ) influenced by co digestion as well as mixing ratio of the substrates. The cumulative average volume of biogas (CAVB) production after 8 WOD is in the order of 50:50 (cow dung : poultry manure) > 25:75 (cow dung : poultry manure) > 0:100 (cow dung : poultry manure) > 100:0 (cow dung : poultry manure) > 75:25 (cow dung : poultry manure). This is similar to observation by Adelekan and Bamgboye (5), who maintained that co-digesting different livestock wastes with cassava peels at a mixing ratio of 1:1 had significant effect in increasing average cumulative biogas yield. They posited that substrates with very high C/N ratio would produce very low biogas (Table 4). However, when co-digested with materials with a low C/N ratio, stabilize the ratio to an optimal value between 22 and 30 (33) which enhance methanogenesis. Li *et al.*, (25), stressed that Co-digestion provides positive synergisms which is mainly attributed to more balanced nutrients and increased buffering capacity, bacterial diversities in different wastes and the supply of missing nutrients by the co-substrates (34). Ofoefule, *et al.*, (22), noted other physicochemical properties like high volatile solids (VS) and sufficient pH range of 6.5 to 8.0 optimization strategies provided by co-digestion to improve biogas production (35, 36). Plant-based biomass is highly lignocellulosic, which inhibit biogas production. Mixing with livestock wastes (poultry, piggery and cattle manure) lowers the C/N ratio of the mixture, making it more digestible, due to more microbial presence (5). Biogas production has been found to be affected by mixing ratio irrespective of biomass waste type. This is because higher mixing ratios meant higher C/N as well as lignin content which could hinder microbial activities and methanogenesis (5). According to Ghasimiet *al.* (37), an excessively high C:N ratio implied an increase in acid formation which retards methanogenesis and methane yield. This could have informed the pattern of yield for treatments with lower C:N values despite their status as co substrates. The 50:50 mixing ratio (treatment C) had the highest biogas yield, which is adduced to the relative low lignin content, moderate C:N closer to the range reported by Karkiet *al.* (33), since it is a mixture of two livestock manures, which therefore substantiated the findings of Adelekan and Bamgboye, (5).

### **3.2.3 Effect of Anaerobic Digestion on Proximate Composition of Samples**

The significant variations in ash and total solid (TS) contents after anaerobic digestion (AD) for all treatments was in line with the findings of Bagudo *et al.*, (38); Akinfemiet *al.* (39) and Ofoefule and Ibeto (22). The total solid content of the wastes had been thought to be comprised of the ash and volatile solid (biodegradable portion of the organic substrate). The degradation of the volatile solid fraction would have resulted in reduction of the TS of the spent slurries. Thus, the volume of biogas generated from the substrates is directly proportional to the difference in the total solid between the substrate and its spent slurry (38). %total lipid (TL) and % Volatile Solid (VS) contents of the spent slurries were reduced for all samples after AD. These were reported to be due to the conversion of the volatile organic matter, which was present in the fresh slurries. This result was in line with earlier claim by Meynell (40) and Ofoefule and Uzodinma (41) who stressed that volatile solids of organic wastes decrease as anaerobes degrade them under anaerobic condition. The reduction in TL has been attributed to its metabolism (42). The increase in % moisture content contrasted the reduction in % moisture content (MC) as observed by Tchobanoglous *et al.*, (43) and Eze and Okonkwo (44). They indicated that the anaerobic process is a net water consuming, as revealed by their studies on some organic wastes. High moisture contents usually facilitate the anaerobic digestion; however, it is difficult to maintain the same availability of water throughout the digestion cycle (45). Initially water added at a high rate is dropped to a certain lower level as the process of anaerobic digestion proceeds. High water contents are likely to affect the process performance by dissolving readily degradable organic matter. It has been reported that the highest methane production rates occur at 60–80% of humidity (46). Hernandez-Berrie *et al.* (45), studied methanogenesis processes during anaerobic digestion at different moisture levels i.e., 70% and 80%. They found that the methanogenesis phase took place around day 70 in both cases, at 70% and 80% moisture. However, bioreactors under the 70% moisture regime produced a stronger leachate and consequently a higher methane production rate.

There variations in % crude protein (CP), % crude fibre (CF) and % nitrogen free extract (NFE) have been attributed to the nature of and mixing ratios of the substrate treatments. The increase in CP content of all the substrates after anaerobic degradation suggested that the initial values were adequate for the process, corroborating the range of values of 11.73-16.68% of CP observed by Ofoefule and Ibeto (22), who blended bambara nut-chaff with cow dung for biogas production. The increase in crude protein (CP) content of degraded organic substrates as observed by many workers (47, 48), has been attributed to release of nitrogenous and non-nitrogenous fractions in addition to microbial single cell protein (SCP), which contribute to overall increase in CP content (13). These enhancements were also added to bioconversion of soluble carbohydrates fractions in the substrates to bacterial protein (49), coupled with the production of different enzymes and biomolecules, which are proteinaceous in nature during the process (50, 51). The reduction in % crude fibre (CF) content varied with treatment substrates, except for those of B and E, where there were recorded increases. These observations were thought to be due to activities of cellulolytic microorganisms contained in the substrates. The fungi have been reported to play a predominant role due to their fibre-degrading tendency (52). Belewu and Belewu (53) added the reduction to the production of various enzymes during the vegetative and reproductive phases. Akinfemiet *al.* (39), opined that type of fungi species as well as nature of the fibre was major determinants for crude fibre fraction reduction. The digestion of fibre fraction was thought to be associated with production of soluble sugar, which increases the energy content of the substrates. Part of this energy is utilized for biogas production, while the residual are converted to microbial protein. This, consequently increased the protein fractions of the resultant effluents (53). Tamara *et al.* (55), had recounted that the digestion of CF usually is a water consuming process. Water is required for solubilization of lignin fraction at the vegetative and reproductive phases. This might have accounted for the decrease in moisture content in the present study.

### **3.2.4 Effects of Anaerobic Digestion on Amino Acid Composition of Samples**

The noticeable variations in essential amino acids (methionine and histidine) and non-essential amino acid (glycine and cysteine) contents for all treatments after anaerobic digestion may be due to the hydrolysis of protein to amino acid fractions as well as synthesis by the bacteria (50; 56). During amino acid biosynthesis, a 5-C intermediate ( $\alpha$ -ketoglutarate) resulting from 6-C glucose metabolism is converted to amino acids in the presence of ammonia. According to Liu, *et al.*, (57), various amino acids, vitamins and minerals that are essential for the growth and metabolism of lactic acid bacteria are utilized, which predominantly, could have accounted for reductions of the specific amino acids. Some amino acids (isoleucine, glutamic acid lysine etc) are synthesized from others, serving as precursors. The relative abundance of these amino acids depends on the concentrations of their precursors. Also the availability of certain amino acids depends on their uptake by microbial strains from the medium. Burkovski, (58), established that amino acid uptake during exponential growth in complex medium is a function of the available % of precursor and whether it is synthesized *de novo* or from proteinic amino acid precursor (59). Mineral element constituents of amino acids are often sourced during microbial amino acid biosynthesis (60, 61). Inorganic N as urea, ammonium or ammonia is assimilated

by some microbes for amino acid synthesis. However, some workers have reported some amino acids (glutamine, glutamate, serine, alanine and asparagine) as nitrogen donors for specific amino acid synthesis (60, 61, 62, 64). During the uptake, N constituent is assimilated as ammonia, pyruvate or  $\alpha$ -ketoglutarate (58), this would definitely affect the residual contents. Phosphorus in bacterial cells occurs in inorganic form, mostly Pi, and in organic form mostly as a component in a number of biomass such as RNA and DNA. It plays a central role in energy metabolism since biochemical energy obtained by the oxidation of substrates is used to synthesize ATP from ADP and Pi. Sulphur is used as a constituent of a large number of biomass components, methionine and cysteine being the most dominant. The main source of sulfur for micro-organisms in nature and in fermentations is the inorganic compound sulfate ( $\text{SO}_4^{2-}$ ). Alternatively, degradation of S-containing amino acids (methionine and cysteine) would ultimately reduce their contents in the resultant effluents (65, 66). The possibilities for amino acid reduction include chemical reactions between amino acids and aldehyde groups present as a result of a Maillard reaction (67), deamination of certain polypeptides, shorter fermentation period has been reported to prevent loss of some amino acids which otherwise become lost due to relatively longer duration of digestion. Vijayan *et al.* (49) recorded an increase in content of most of the amino acids at short fermentation period. Safari *et al.* (68), opined that the dominant microbes in the digestion medium (especially for naturally fermented medium) would determine resultant amino acids. Fungal dominated medium would result in higher lysine content while bacterial-rich medium would produce higher methionine content. This is the case as observed in the current study.

#### IV. Conclusion

The process of anaerobic digestion of the different substrates engendered enhancements of some proximate and amino acid compositions such as percentage moisture content, ash crude protein, while crude fibre, total solid and volatile solid content of the digested materials decreased. There were percentage increases in histidine, glycine, cysteine and methionine contents, and variations of other amino acids due anaerobic digestion of the wastes. The co-digested wastes materials gave relatively higher average volume of biogas generation than the single substrates. Thus, revealing the huge industrial and environmental potentials of the process.

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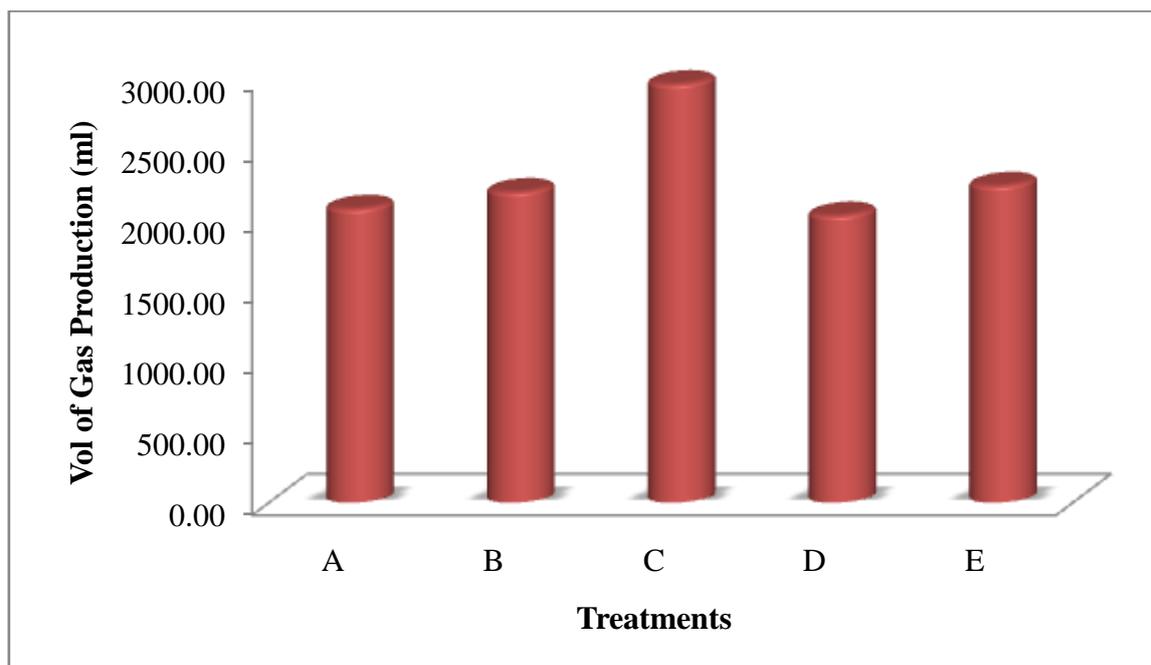
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**Table2:** Average Volume of Gas Production (ml/wk) during the eight weeks Anaerobic Digestion

Trts	Week								Mean
	One	Two	Three	Four	Five	Six	Seven	Eight	
A	66.7±6.1 <sup>bc</sup>	110.0±20.0 <sup>b</sup>	177.3±26.1 <sup>bc</sup>	320.7±20.1 <sup>cde</sup>	358.0±15.1 <sup>b</sup>	393.0±8.5 <sup>b</sup>	381.3±13.0 <sup>cd</sup>	272.0±11.1 <sup>d</sup>	259.88
B	93.3±4.2 <sup>ef</sup>	150.7±19.0 <sup>c</sup>	262.7±16.6 <sup>gh</sup>	316.3±15.0 <sup>cde</sup>	382.3±12.5 <sup>bc</sup>	423.3±14.0 <sup>bc</sup>	385.0±7.0 <sup>cd</sup>	184.3±12.1 <sup>b</sup>	274.74
C	98.3±3.5 <sup>f</sup>	176.7±10.4 <sup>d</sup>	280.3±6.8 <sup>h</sup>	345.7±17.8 <sup>e</sup>	447.3±45.5 <sup>ef</sup>	621.0±39.7 <sup>f</sup>	562.0±12.0 <sup>h</sup>	429.7±26.6 <sup>h</sup>	370.13
D	62.0±11.1 <sup>b</sup>	105.0±3.0 <sup>b</sup>	214.0±4.0 <sup>def</sup>	304.7±5.0 <sup>cd</sup>	376.7±16.1 <sup>bc</sup>	415.7±6.0 <sup>bc</sup>	314.0±12.0 <sup>a</sup>	239.0±4.6 <sup>c</sup>	253.89
E	86.7±2.9 <sup>def</sup>	150.0±5.0 <sup>c</sup>	221.7±6.5 <sup>ef</sup>	315.7±8.0 <sup>cde</sup>	396.7±14.0 <sup>cd</sup>	462.3±11.2 <sup>d</sup>	345.3±11.4 <sup>b</sup>	263.3±11.0 <sup>cd</sup>	280.21

Means along each column bearing different superscripts are significantly different ( $P < 0.05$ ) at 5% level



**Figure 1:** Effect of Anaerobic Digestion on Cumulative Biogas Production

**Table 3:** Proximate Composition of Samples before and after Anaerobic Digestion (% dry matter)

Proximate Composition		A	B	C	D	E
ASH	Before	23.71	30.69	30.19	24.46	29.84
	After	36.97	45.44	45.03	35.60	43.73
	% Diff	<b>55.93</b>	<b>48.06</b>	<b>49.16</b>	<b>45.54</b>	<b>46.55</b>
TL	Before	13.81	6.88	8.49	11.83	6.76
	After	1.82	2.34	3.97	2.16	4.03
	% Diff	<b>-86.82</b>	<b>-65.99</b>	<b>-53.24</b>	<b>-81.74</b>	<b>-40.38</b>
CF	Before	29.85	10.68	24.54	16.04	7.42
	After	22.16	18.80	17.91	9.07	14.06
	% Diff	<b>-25.76</b>	<b>76.03</b>	<b>-27.02</b>	<b>-43.45</b>	<b>89.49</b>
NFE	Before	15.95	31.31	16.75	29.90	45.16
	After	25.69	25.66	21.52	39.99	30.66
	% Diff	<b>61.07</b>	<b>-18.05</b>	<b>28.48</b>	<b>33.75</b>	<b>-32.11</b>
CP	Before	12.13	16.19	15.56	13.25	6.25
	After	19.19	15.26	19.40	16.96	15.31
	% Diff	<b>58.20</b>	<b>-5.74</b>	<b>24.68</b>	<b>28.00</b>	<b>144.96</b>
MC	Before	4.55	4.25	4.47	4.52	4.57
	After	22.17	15.51	28.18	26.23	28.22
	% Diff	<b>387.25</b>	<b>264.94</b>	<b>530.43</b>	<b>480.31</b>	<b>517.51</b>
TS	Before	95.45	95.76	95.53	95.48	95.43
	After	77.83	84.49	71.82	73.77	71.78
	% Diff	<b>-18.46</b>	<b>-11.77</b>	<b>-24.82</b>	<b>-22.74</b>	<b>-24.78</b>
VS	Before	71.74	65.07	65.34	71.02	65.59
	After	40.86	39.05	26.79	38.17	28.05
	% Diff	<b>-43.04</b>	<b>-39.99</b>	<b>-59.00</b>	<b>-46.25</b>	<b>-57.23</b>

TL = Total lipid, CF = Crude Fiber, NFE = Nitrogen Free Extract, CP = Crude Protein, MC = Moisture Content, TS = Total Solid, VS = Volatile Solid

**Table 4:** Carbon/Nitrogen contents of Samples Before and After Anaerobic Digestion

Trt	C/N <sub>Before AD</sub>	C/N <sub>After AD</sub>	%C/N <sub>Red</sub>
A	18.43	13.02	29.35
B	14.30	12.45	12.94
C	12.82	7.93	38.38
D	21.38	11.59	43.65
E	20.60	8.39	59.27

C/N<sub>Before AD</sub> = Carbon/Nitrogen contents of Samples before anaerobic digestion

C/N<sub>After AD</sub> = Carbon/Nitrogen contents of Samples after anaerobic digestion

%C/N<sub>Red</sub> = Percentage Carbon/Nitrogen reduction after anaerobic digestion

A = 100:0 cow dung, B = 100:0 Poultry manure, C = 50:50 cow dung + poultry manure, D = 75:25 cow dung + poultry manure, E = 25:75 cow dung + poultry manure

**Table 5:** Amino Acid Contents of Samples before and after Anaerobic Digestion (g/100g of protein)

Amino Acids	A			B			C			D			E		
	Before	After	% Diff												
Lys	3.50	2.03	-42.00	2.83	2.00	-29.33	2.91	2.46	-15.46	1.82	2.00	9.89	3.02	1.97	-34.77
His	1.09	5.27	383.49	1.35	4.01	197.04	1.37	4.38	219.71	1.15	6.25	443.48	1.55	5.27	240.00
Arg	1.71	1.90	11.11	3.02	2.24	-25.83	2.05	1.99	-2.93	2.30	1.99	-13.48	2.33	1.81	-22.32
Asp	4.02	2.79	-30.60	4.05	2.26	-44.20	4.65	2.79	-40.00	3.20	2.64	-17.50	4.20	2.78	-33.81
Thr	1.34	2.31	72.39	2.16	2.01	-6.94	1.42	2.15	51.41	1.91	2.21	15.71	1.28	1.99	55.47
Ser	1.93	1.25	-35.23	1.93	1.14	-40.93	2.07	1.30	-37.20	1.71	1.60	-6.43	2.31	1.49	-35.50
Glu	6.21	7.57	21.90	7.23	5.68	-21.44	6.01	7.80	29.78	5.40	6.89	27.59	7.10	8.26	16.34
Pro	1.17	2.24	91.45	2.18	2.14	-1.83	1.45	2.44	68.28	1.26	2.03	61.11	1.39	2.34	68.35
Gly	2.75	7.25	163.64	3.05	6.89	125.90	3.08	5.60	81.82	3.32	6.01	81.02	2.96	5.00	68.92
Ala	2.23	3.34	49.78	3.55	3.08	-13.24	2.55	3.46	35.69	3.30	2.81	-14.85	2.80	2.81	0.36
Cys	0.51	3.97	678.43	0.57	4.43	677.19	0.77	3.77	389.61	0.58	3.51	505.17	1.03	3.18	208.74
Val	2.60	2.52	-3.08	3.50	2.35	-32.86	3.00	2.37	-21.00	2.70	2.00	-25.93	2.83	1.91	-32.51
Met	0.41	0.63	53.66	0.46	0.63	36.96	0.36	0.70	94.44	0.40	0.44	10.00	0.51	0.63	23.53
Ileu	1.73	2.00	15.61	2.25	1.87	-16.89	2.16	2.22	2.78	1.63	2.00	22.70	1.79	1.87	4.47
Leu	2.84	3.20	12.68	7.25	2.89	-60.14	3.00	3.16	5.33	2.50	3.00	20.00	2.71	2.98	9.96
Tyr	1.08	1.27	17.59	2.36	1.91	-19.07	1.61	1.59	-1.24	1.69	1.43	-15.38	1.25	2.06	64.80
Phe	2.30	1.85	-19.57	3.90	2.19	-43.85	2.25	2.19	-2.67	2.75	2.11	-23.27	3.21	2.45	-23.68
TAA	37.51	51.39	37.00	51.64	47.72	-7.59	40.71	50.37	23.73	37.62	48.92	30.04	42.27	48.80	15.45
TEAA	17.52	21.71	23.92	26.72	20.19	-24.44	18.52	21.62	16.74	17.16	22.00	28.21	19.23	20.88	8.58
TNEAA	19.99	29.68	48.47	24.92	27.53	10.47	22.19	28.75	29.56	20.46	26.92	31.57	23.04	27.92	21.18
TSAA	0.92	4.60	400.00	1.03	5.06	391.26	1.13	4.47	295.58	0.98	3.95	303.06	1.54	3.81	147.40
TArAA	3.38	3.12	-7.69	6.26	3.38	-46.01	3.86	3.78	-2.07	4.44	3.54	-20.27	4.46	4.51	1.12