Impact of organic and inorganic fertilizers on yield and quality of silage corn in intercropped system with soybean

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Corn silage is an important feed for intensive ruminant production but the growing of corn has relied heavily on the use of chemical fertilizer. Sustainable crop production requires a careful management of all nutrient sources available in a farm, particularly in corn-based cropping systems. Experiments were conducted to determine the appropriate technology of corn-legume intercropping with supplemental use of chemical, organic manure, and biofertilizer. Combining chemical fertilizers with chicken manure in a 50:50 ratio and application of 50% NPK+ 50%, chicken manure (CM)+ biofertilizer (BF) resulted in similar dry matter (DM) yield with the 100% NPK treatment. Inorganic fertilizer (100% NPK) gave the highest DM yield (13.86 t/ha) of forage among single fertilizer treatments and it outyielded the chicken manure (100% CM) (9.74 t/ha) treatment. However, when CM was combined with NPK, the DM yield of forage (13.86 t/ha) and was the same as the 100% NPK (13.68 t/ha). Combinations of NPK and chicken manure resulted in increased plant height; crop growth rate (CGR) and leaf area index (LAI) compared to CM alone but was similar to 100% NPK application. The ratio of 50% CM + 50% NPK and 50%CM+50%NPK+BF recorded protein yield similar to those of conventional fertilizer. Similarly, CP content was not significantly different among 100% NPK and 50% CM+50% NPK. Use of biofertilizers had no significant impact on improving either yield or quality of forage fertilized with inorganic or organic fertilizers. Lactic acid responded differently to different fertilizer application and was significantly higher than the no fertilizer plots. Treatments with an application of biofertilizer and combination of biofertilizer with NPK or CM treatments gave higher values of acetylene reduction assay (ARA) (compared to sole chemical and sole organic manure fertilizers. Overall, evidence recorded from this study prove that corn-soybean intercrops could increase forage quantity and quality, produce higher total protein yield, decrease requirements for protein supplements and chemical fertilizer compared to the corn monoculture with a combination of chicken manure and chemical fertilizer.

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Impact of organic and in-organic fertilizers on yield and quality of silage corn in intercropped system with soybean

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ABSTRACT

Corn silage is an important feed for intensive ruminant production but the growing of corn has relied heavily on the use of chemical fertilizer. Sustainable crop production requires a careful management of all nutrient sources available in a farm, particularly in corn-based cropping systems. Experiments were conducted to determine the appropriate technology of corn-legume intercropping with supplemental use of chemical, organic manure, and biofertilizer. Combining chemical fertilizers with chicken manure in a 50:50 ratio and application of 50% NPK+ 50%, chicken manure (CM)+ biofertilizer (BF) resulted in similar dry matter (DM) yield with the 100% NPK treatment. Inorganic fertilizer (100% NPK) gave the highest DM yield (13.86 t/ha) of forage among single fertilizer treatments and it outyielded the chicken manure (100% CM) (9.74 t/ha) treatment. However, when CM was combined with NPK, the DM yield of forage (13.86 t/ha) and was the same as the 100% NPK (13.68 t/ha). Combinations of NPK and chicken manure resulted in increased plant height; crop growth rate (CGR) and leaf area index (LAI) compared to CM alone but was similar to 100% NPK application. The ratio of 50% CM + 50% NPK and 50%CM+50%NPK+BF recorded protein yield similar to those of conventional fertilizer. Similarly, CP content was not significantly different among 100% NPK and 50% CM+50% NPK. Use of biofertilizers had no significant impact on improving either yield or quality of forage fertilized with inorganic or organic fertilizers. Lactic acid responded differently to different fertilizer application and was significantly higher than the no fertilizer plots. Treatments with an application of biofertilizer and combination of biofertilizer with NPK or CM treatments gave higher values of acetylene reduction assay (ARA) (compared to sole chemical and sole organic manure fertilizers. Overall, evidence recorded from this study prove that corn-soybean intercrops could increase forage quantity and quality, produce higher total protein yield, decrease requirements for protein supplements and chemical fertilizer compared to the corn monoculture with a combination of chicken manure and chemical fertilizer.

Keywords: inorganic fertilizer, biofertilizer, volatile fatty acids, fermentation analysis, corn silage, , crude protein, intercropping, nutritive quality, biological nitrogen fixation (BNF), acetylene reduction assay

INTRODUCTION

Feed products from corn are characterized by high-energy content, but relatively low content of crude protein with low biological value (Summers, 2001; Mlynár et al., 2004). Corn forage, with its relatively high-energy content, is also well adapted for use in low cost rations for fattening livestock. Furthermore, corn forage can efficiently recycle plant nutrients, especially large amounts of N, P and K (Roth and Heinrichs, 2001).

Corn productivity in tropical low external input systems is usually limited by low soil fertility because crop uptake leads to a gradual depletion of soil nutrient stocks. Since the use of chemical fertilizers is undesirable, the management of the fertilizer of these soils depends primarily on low cost processes based on nutrient recycling (Figueiredo et al., 2009). The main processes that may contribute to this are biological nitrogen fixation (BNF) and nutrient recycling through organic fertilization. Biological nitrogen fixation may contribute to corn growth and yield by direct fixation in corn, or through the use of legume plants either as crops in rotation or intercropped with corn. Either way, BNF can usually be considered as long-term sustainable nitrogen source for low external input corn production systems (Figueiredo et al., 2009).

Being a fast-growing plant with C_4 photosynthesis, corn requires plentiful supply of the essential elements nitrogen, phosphorus and potassium, which traditionally have been obtained by inputs of chemical fertilizers to replenish soil nitrogen and phosphorus, resulting in high costs and environmental pollution (Dai et al., 2004; Awodun et al., 2007). The harmful effects on the environment of heavy use of nitrogen fertilizer are becoming more evident. There is a need for sustainable farming, which maintains soil fertilizer by using renewable resources easily and cheaply available on the farm. The supply of other nutrients such as phosphorus can also be enhanced with the use of biofertilizers (Mayer et al., 2008; Oliveira et al., 2009). Organic fertilizers including farmyard manure, chicken manure, sheep manure, and biofertilizer may be used for crop production as a substitute of chemical fertilizers (Khan et al., 2005). Organic fertilizers improve soil fertility without leaving any residual effects in the soil and are much cheaper as compared to chemical fertilizers (Chater and Gasser, 1970).

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Soybean crop is capable of supplying nitrogen for its growth and intercropped cereals through symbiotic nitrogen fixation, and hence reduces the need for expensive and environment polluting nitrogen fertilizer (Rochester et al., 2001). More studies are needed to fully understand the extent to which the nitrogen requirements of soybean grown at potential yields levels can be met by optimizing BNF alone as opposed to supplementing BNF with applied nitrogen (Salvagiotti et al., 2008).

The intercropping system greatly contributes to crop production by its effective utilization of resources, as compared to the sole cropping system (Inal et al., 2007). The major factor contributing to declining crop yields is a soil fertilizer reduction caused by continuous cropping without the addition of sufficient mineral fertilizers and manures (Ndayisaba, 2014). The combined application of chemical and organic sources, usually termed integrated nutrient management, is widely recognized as a way of increasing crop productivity sustainably (Mahajan et al., 2008).

The need to decline costs of fertilizing crops with renewable forms of energy has revitalized the application of organic fertilizers around the world. The main reasons for promoting enhanced use of organic materials are improvement of environmental conditions and public health (Ojeniyi, 2000; Maritus and Vlelc, 2001).

Nutrients from the organic manures are supplemented with inorganic nutrients that are readily available to plants (Ayoola and Makinde, 2008). Nutrients are released more slowly from organic manure and they are stored for a longer time in the soil, thus ensuring a long residual effect (Sharma and Mittra, 1991; Abou el Magd et al., 2005). Chicken manure was found to be viable substitutes for chemical fertilizers (Khan et al., 2005) and indicated a strong positive effect on moisture holding capacity and structure of the soil (Sharif et al., 2004). Biofertilizers could assist to increase efficiency of BNF through production of plant growth promoting substances (Kucey, 1989).

Integrated use of fertilizer sources helps to maintain the fertility status of the soil. Ghosh, et al. (2004) reported application of 75% NPK in combination with poultry manure (PM) or Farmyard manure or phosphocompost in sorghum and soybean crops and 75% NPK in wheat caused in significantly higher grain yields of wheat than those in inorganic and control and saved 25% NPK fertilizer as a result of additional production of following wheat crop. Within the cropping systems, soybean as preceding crop gained the

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maximum seed yield of wheat and had not a significant difference with that of soybean/sorghum intercropping system.

However, integration of modest amounts of inorganic fertilizers with organic materials such as chicken manure, biofertilizer and chemical, offers a strategy to meet smallholder crop nutrient requirements, especially in nutrient intensive corn-legume intercrop systems. It seems that silage yield and quality will not reduced by intercropping even with reduction in the level of NPK fertilizer application. The objective of the experiment therefore, was to evaluate the organic and/or inorganic fertilizer application on improvement of quality and quantity in forage corn intercropped with soybean in relation to DM yield, nutritive value and volatile fatty acids production.

MATERIALS AND METHODS

Treatments and experimental design

The experiment was conducted in the agronomy field, University Putra Malaysia, located at latitude 3: 02' N longitude of 101: 42' E, in a sandy loam soil with an organic carbon 3.02%, and pH of 6.03. Total annual rainfall in the year 2014 was approximately 2689 mm with a monthly average of 224.08 mm. Peak rainfall was recorded in November (333.1 mm) with the least in June (139.4 mm). Mean monthly minimum and maximum temperatures ranged from 24.3°C to 26.2°C and 31.5°C to 35.1°C respectively, while average monthly relative humidity ranged from 91.5 to 94.7%.

The treatments consisted of ten-fertilizer application (The list of treatments is presented in Table 1). The intercrop composition was based on replacement design. Experiments were arranged as a randomized complete block design with four replications. A silage corn cultivar (926) and a local soybean (Glycine max) cultivar were utilized in the current research study.

The biofertilizer preparation was conducted in the Soil Microbiology Laboratory, Department of Land Management of Universiti Putra Malaysia. The bio-fertilizer used in this study contained a consortium of nitrogen fixing bacteria (SB13, SB16, SB26, SB35, SB42) and phosphate solubilizing bacteria (PSB) (PSB16). For each bacteria, a 1 liter solution comprising 8 g nutrient broth (NB) and 1 liter distilled water in a flask was prepared. The flasks were kept in autoclave for 2 hours and transferred in laminar flow (40 ^{o}C in 30 minutes. 1-2 full loops of 1 strain of bacteria were added in flasks. All six flasks were placed in shaker for 72 hours (precipitation occurred) and

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mixed in one flask and was placed in shaker for another 72 hours. Seeds of corn and legumes (for biofertilizer treatments) were soaked in biofertilizer solution for 90 minutes and seeds were used for planting. After the seeds had germinated (1 week), the solution of biofertilizer was applied in biofertilizer treatments in the field. For 5 liter biofertilizer solution 4.9 liter of 2.5% molasses solution prepared in the incubation tank and stock added and incubated for 2 days until the population become 10^9 cfu/ml (Panhwar et al., 2014). Biofertilizer volume needed sprayed per area basis (10 ml/plant). Based on soil analysis and chicken manure analysis the required level of chicken manure was 10 t/ha that was applied before planting. All other agronomic practices were kept at a uniform level.

Plant sampling, plant growth, yield and yield components

Corn and soybean intercrops were harvested at the same time. Corn was harvested when the kernel milk-line was between 50 and 75% and for soybean at seed fill stage. They were weighed fresh to determine fresh forage yield. The sampled area was 5 m2 for the monoculture corn and intercropping treatments at the center of each plot and fresh biomass weight was determined as g DM m-2 and above plant parts were harvested by hand cutting the plant 2 cm above the soil surface. Samples were oven-dried at 70°C for at least 72 hours. Forage DM yield was calculated from the fresh and dry weights of respective components listed above. Prior to harvest, measurements of parameters such as leaf area index (LAI), photosynthesis and leaf chlorophyll were taken. The equipment used respectively were LICOR LAI-2000 Plant Canopy Analyzer, LICOR LI-6400 portable photosynthesis system Lincoln Nebraska USA, LICOR Minolta SPAD-502 chlorophyll meter.

Nutritive quality measurements

Sample plants of each plot were chopped and mixed mechanically, and a 500 g sub-sample of each weighed forage sample was dried for 7 days in a 70 oC forced-air oven to constant moisture to determine forage quality characteristics. Dried sub-samples were retained for forage quality assays. All dried samples were ground using a hammer mill to pass a 1-mm screen and analyzed for nutritive quality values. Quality of forages analyzed using NIRS (near infrared spectroscopy) (Jafari et al., 2003). NIRS analyses requires a sample (0.5-1.0 g) which is exposed to an electro-magnetic scan over a

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spectral wavelength range of 1100 to 2500 nm (near infrared). Energy in this spectral range is directed on to the sample and reflected energy (R) is measured by the instrument. The diffuse reflection carries information, which identifies chemical bonds within the sample, such as -CH, -OH, -NH and -SH. The reflected energy is stored as the reciprocal logarithm (log 1/R) and the spectra are transformed to provide information about the chemical composition of the sample (Baker and Barnes, 1990; Shenk and Westerhaus, 1993).

Determination of fermentation characteristics and NH_3-N

Silage preparation with an Otosilager® (Forage compacting machine) described by Baghdadi et al. (2016). After 10 weeks, the silos were opened and representative samples were taken for analysis. Gas chromatography (GC) is preferred method for volatile fatty acids measurement. VFA was determined using an Agilent 7890A gas-liquid chromatography (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame ionization detector (FID). For determination of VFA and lactic acid, methyl n-valeric acid and fumaric acid as the internal standard used respectively (Supelco, 1990; procedures cited from Lombard and Dowell, 1962). Figure 1 shows processes of silage fermentation analysis. A pH electrode (Mettler-Toledo Ltd., England) was used to determine the pH of silage after the sample preparation. Ammonium nitrogen was determined in corn-soybean silage samples using the colorimetric method described by Solorzano (1969).

Determination of biological nitrogen fixation (BNF)

The discovery that the nitrogenase enzyme responsible for N-fixation also reduced C_2H_2 (acetylene) to C_2H_4 (ethylene) (Dilworth, 1966) provided a useful assay for the quantification of the N-fixation process. However, the acetylene reduction assay (ARA) is still used widely because it provides a highly sensitive and inexpensive way to quantify relative nitrogenase enzyme activity in N fixing samples. Because the presence of acetylene blocks the conversion of N_2O to N_2, we are able to simultaneously measure denitrification (NO_3- $\rightarrow N_2O \rightarrow N_2$) by measuring N_2O.

Nitrogen fixation was estimated during vegetative growth (R_5 stage of soybean-30 days after planting). Nodulated roots of soybean plants and roots of corn plants were placed in a 1000 ml incubation vessel (PVC wide mouthed bottle). After the cap of the incubation vessel was secured tightly, 50 ml (5%) of the air was withdrawn from the incubation vessel (via the rubber septum in the cap) with a 50 ml plastic syringe and replaced with 50 ml of C_2H_2. After one h incubation, acetylene production was measured by injecting 1 mL of the headspace gas from each of the syringes into a gas chromatograph (Agilent 5890 Series Gas Chromatograph, Wilmington, DE, USA) equipped with FID detector. Separation was achieved using an HP-Plot Q column (30 m × 0.53 mm × 40 m) (Agilent Technologies, Wilmington, DE, USA) with nitrogen (99.9% purity, Domnick-Hunter generator, Domnick-Hunter, Leicester, UK) as the carrier gas at the flow rate of 3.5 mL/min. An iso-thermal oven temperature of 50 ^{o}C was adopted in the separation (Figure 2). Calibration was completed using standard ethylene prepared by Scotty Specialty Gases (Supelco, Bellefonte, PA, USA). All the procedures were repeated three times. Ethylene C_2H_4 produced by the nodules was calculated (use by values of the standard calculated previously from the calibration of the gas chromatograph.

Statistical analysis

The results were statistically processed using analysis of variance (ANOVA) with the Statistical Software System (SAS) (Version 9.1). The Least Significant Difference (LSD) was used to compare treatment means at the 0.01 and 0.05% probability levels.

RESULTS

Yield and DM yield of corn-legume intercropping

Dry matter yield is a measure of forage productivity. Corn and soybean yield were higher than control in all fertilizer treatments ($P<0.01$). Among the three sole fertilizers 100% NPK gave significantly higher total forage DM yield (13.86 t/ha) than 100% chicken manure (9.74 t/ha) which was also significantly higher than biofertilizer (6.72 t/ha). Application of 50% NPK+50% CM gave similar DM yield (13.68 t/ha) with 100% NPK which implies that CM can substitute for half of NPK without affecting DM yield. Application of 50% NPK+50% CM gave higher DM yield (13.68 t/ha) than 100% CM fertilizer treatment (9.74 t/ha) (Figure 3).

No significant response of forage DM yield to biofertilizer application was observed though the combination with NPK and CM. Application of 50% NPK gave same DM yield (9.67 t/ha) as 100% CM (9.74 t/ha). DM yield

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from 100% NPK fertilizer application was significantly higher than yield from 50% NPK fertilizer application. Similarly, use of 100% chicken manure gave significantly higher DM yield (9.74 t/ha) than fertilizer application of 50% CM (6.75 t/ha). Results showed that application of 50% NPK fertilizer or 50% chicken manure alone could not full fill the nutrient requirement of plants. A combination of organic manure, chemical and biofertilizer resulted in increase in forage DM yield over control but was similar with 100% NPK application and 50% NPK+50% CM treatment.

Plant growth characteristics

Among the sole fertilizer, 100% NPK gave significantly taller plant height of corn (197 cm) than 100% chicken manure (184 cm) which was also significantly higher than biofertilizer (152.50 cm) treatment. Similarly, 100% NPK showed taller plant height of soybean (109.25 cm) than 100% chicken manure (99 cm) which was also significantly higher than and biofertilizer (76.50 cm) ($P<0.01$) (Table 2).

The integration of chemical fertilizer with chicken manure increased corn and soybean plant height to the same level as sole inorganic fertilizer. Application of 50% NPK+50% CM gave same corn plant height (195.25 cm) as sole NPK. Additionally, fertilizer treatment of 50% NPK+50% CM gave same soybean plant height (112.75 cm) as 100% NPK. Plant height of corn and soybean was significantly taller through a combined application of chemical fertilizer and chicken manure compared to 100% chicken manure.

Corn and soybean CGR and LAI were significantly higher than control for all fertilizer treatments ($P<0.01$). Higher value of corn CGR was obtained with 100% NPK (29.34 g/m^2/day) compared to 100% CM (26.37 g/m^2/day) and sole biofertilizer (22.51 g/m^2/day). Similarly, among the three sole fertilizers 100% NPK gave significantly higher soybean CGR (9.55 g/m^2 /day) than 100% CM (8.48 g/m^2/day) which was also significantly higher than BF (7.75 g/m^2/day).

Application of 50% NPK+50% CM gave same corn CGR (28.98 g/m^2/day) as sole NPK. Additionally, fertilizer treatment of 50% NPK+50% CM gave same soybean CGR (9.39 g/m^2/day) as that of 100% NPK. Results showed that CGR values of both crops were significantly higher through a combined application of NPK and CM compared to 100% CM.

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Among sole fertilizer applications 100% NPK gave, the highest LAI of corn plant (2.56) followed by 100% chicken manure (2.39) and biofertilizer (2.15). In addition, sole NPK gave significantly higher soybean LAI (3.17) than sole CM (83.07) which was also significantly higher than sole biofertilizer (2.78). Combination of NPK and chicken manure fertilizer increased corn and soybean LAI to same level as sole NPK. Application of 50% NPK+50% CM gave same corn LAI (2.52) as sole NPK. Similarly, treatment of 50% NPK+50% CM gave same soybean LAI (3.20) as 100% NPK. Corn and soybean LAI in combined application of NPK and CM was significantly higher than that of 100% CM. A combination of biofertilizer, organic manure and chemical fertilizer resulted in increase in corn and soybean LAI over control but was similar with 100% NPK application and 50% NPK+50% CM treatment.

Corn-legume forage nutritive quality

Results showed that CP concentration of corn-soybean forage was markedly affected by different fertilizer treatments. All fertilizer application had significantly higher mean CP percentages than the control treatment ($P<0.01$). Among sole fertilizer application mean CP percentage was not significantly different between that of 100% NPK (14.48%) and 100% CM (14.50%), but they were significantly higher than those of biofertilizer application (13.02%) (Table 3).

Combination of chemical fertilizer and chicken manure increased mix forage CP concentration to same level as sole chemical fertilizer. Application of 50% NPK+50% CM gave same CP content (14.55%) as 100% NPK which implies that CM can substitute for half of NPK to obtain good quality of forage without affecting DM yield. The integration of chemical fertilizer and chicken manure increased CP content to same level as sole chicken manure. Application of 50% NPK+50% CM gave same CP content (14.55%) as 100% CM.

No significant response of mix forage CP concentration to biofertilizer application was observed though the integration with NPK. Application of 50% NPK+BF gave same CP content (13.45%) as 50% NPK (13.33%). Similarly, no significant response of forage CP content to biofertilizer application was observed though the combination with CM. Treatment of 50% CM+BF did not increase CP concentration to same level as 100% CM.

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Application of 50% CM+BF gave same CP content (13.38%) as 50% CM (13.28%). There was no observed positive effects of bio-fertilizer on forage CP concentration in combination with NPK or chicken manure.

Corn-soybean forage CP from 100% NPK was significantly higher than CP from 50% NPK fertilizer application. Similarly, use of 100% chicken manure gave significantly higher CP than fertilizer application of 50% CM. Results showed that application of 50% NPK fertilizer or 50% chicken manure alone could not improve mix forage quality because of CP concentration. Integration of biofertilizer, organic manure and chemical fertilizer resulted in increase in forage CP concentration over control but was similar with 50% NPK+50% CM and 100% NPK application.

Among sole fertilizer application, NDF concentration was not significantly different between that of 100% NPK (57.34%) and sole biofertilizer (58.13%), but they were significantly higher than those of sole chicken manure application (49.22%). Similarly, ADF content was not significantly different between that of 100% NPK (35.68%) and biofertilizer (36.09%), but they were significantly higher than those of 100% CM application (26.66%). Application of 50% NPK+50% CM gave lower NDF content (49.67%) than NDF content with 100% NPK treatment. In addition, combination of 50% NPK+50% CM gave lower ADF content (26.79%) than that of 100% NPK application. The combination of chemical fertilizer and chicken manure decreased NDF and ADF concentration to same level of sole chicken manure. Application of 50% NPK+50% CM gave same NDF (49.67%) and ADF concentration (26.79%) as 100% chicken manure.

No significant response of forage NDF and ADF concentration to biofertilizer application was observed though the integration with NPK. Application of 50% NPK+BF gave same NDF (58.52%) as 50% NPK (57.73%). In addition, combination of 50% NPK+BF gave same ADF (36.12%) as 50% NPK (35.30%). Similarly, no significant response of forage NDF and ADF content to biofertilizer application was observed though the combination with CM. Application of 50% CM+BF gave same NDF content (58%) as 50% CM (57.39%). Additionally, treatment of 50% CM+BF gave same ADF content (36.17%) as 50% CM (35.30%). There was no observed

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positive effects of bio-fertilizer on forage concentration of NDF and ADF in combination with NPK or chicken manure.

Application of 50% NPK gave same NDF content (57.73%) and ADF content (35.30%) as 100% NPK. In addition, corn-soybean forage NDF content from 100% CM (49.22%) fertilizer application was significantly lower than NDF from 50% CM (57.39%) application. Similarly, use of 100% chicken manure gave significantly lower ADF content (26.66%) than fertilizer application of 50% CM (35.23%). A combination of chemical, organic manure and biofertilizer resulted in reduced NDF and ADF concentration over control but was similar with 100% chicken manure treatment and 50% NPK+50% CM application.

Results showed that CP yield of corn-soybean forage was markedly affected by different fertilizer treatments (Table 3,4). All fertilizer application had significantly higher mean CP yield than the control treatment (P<0.01). Among sole fertilizer application mean CP yield was not significantly different between that of 100% NPK (2009.98 kg/ha) and 100% CM (1408.95 kg/ha), but they were significantly higher than those of biofertilizer application (894.25 kg/ha).

Integration of chemical fertilizer and chicken manure increased mixed forage CP yield to same level of sole chemical fertilizer. Application of 50% NPK+50% CM gave same CP yield (1991.95 kg/ha) of 100% NPK which implies that CM can substitute for half of NPK to produce high protein yield forage without affecting DM yield. Application of 50% NPK+50% CM gave higher CP yield (1991.95 kg/ha) than 100% CM fertilizer treatment (1408.95 kg/ha).

No significant response of forage CP yield to biofertilizer application was observed though the combination with NPK. Application of 50% NPK+BF gave same CP yield (1307.87 kg/ha) as 50% NPK (1288.39 kg/ha). Similarly, no significant response of forage CP yield to biofertilizer application was observed though the combination with CM. Treatment of 50% CM+BF did not increase CP yield to the same level of 100% CM. Application of 50% CM+BF gave same CP yield (923.74 kg/ha) as 50% CM (896.03 kg/ha).

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There were no positive effects of bio-fertilizer observed on forage CP yield in combination with NPK or chicken manure.

Corn-soybean forage CP yield from 100% NPK fertilizer application (2009.98 kg/ha) was significantly higher than CP yield from 50% NPK (1288.39 kg/ha) application. Similarly, use of 100% chicken manure gave significantly higher CP yield (1408.95 kg/ha) than fertilizer application of 50% CM (896.03 kg/ha). Results showed that application of 50% NPK fertilizer or 50% chicken manure alone could not full fill the nutrient requirement of plants for CP yield production. A combination of organic manure, chemical and biofertilizer resulted in increase in forage CP yield over control but was similar with 100% NPK application and 50% NPK+50% CM treatment.

Chemical, chicken manure, biofertilizer and integrated of these fertilizers effects were not significant for WSC concentration where WSC of forage ranged from 22.02 to 22.73%. Additionally, application of different fertilizer treatments effects were not significant for DMD which ranged from 65.69 to 66.98%. Furthermore, sole and combination fertilizer treatments effects were not significant for ADL concentration where ADL of forage ranged from 3.62 to 3.80%.

Volatile fatty acids in silage

Among sole fertilizer application, lactic acid content was not significantly different between that of 100% NPK (4.04%) and sole chicken manure (4.02%), but they were significantly higher than those of sole biofertilizer application (3.91%). Similarly, ammonia-N content was not significantly different between that of 100% NPK (2.06%) and 100% chicken manure (2.07%), but they were significantly higher than that of sole biofertilizer (1.84%) treatment (Table 4).

Integration of NPK and chicken manure increased mixed silage lactic acid and ammonia-N concentration to same level as sole chemical fertilizer. Treatment of 50% NPK+50% CM gave same lactic acid content (4.01%) as 100% NPK. Likewise, application of 50% NPK+50% CM gave same ammonia-N concentration (2.08%) as 100% NPK application. The combination of chemical fertilizer and chicken manure increased lactic acid and ammonia-N content to same level as sole chicken manure. Application of 50% NPK+50% CM gave same lactic acid content (4.01%) as 100% CM. In addition, application of 50% NPK+50% CM gave same ammonia-N concentration as 100%NPK treatment.

No significant response of silage lactic acid and ammonia-N concentration to biofertilizer application was observed though the integration with chemical fertilizer. Treatment of 50% NPK+BF gave same lactic acid content (3.93%) as 50% NPK (3.92%). Additionally, application of 50% NPK+BF gave same ammonia-N content (1.83%) as 50% NPK (1.83%).

No significant response of silage lactic acid and ammonia-N concentration to biofertilizer application was observed though the integration with chicken manure. Application of 50% CM+BF gave same lactic acid content (3.92%) as 50% CM (3.92%). Additionally, application of 50% CM+BF gave same ammonia-N content (1.83%) as 50% CM (1.83%) treatment. There were no positive effects of bio-fertilizer observed on silage lactic acid and ammonia-N concentration in combination with NPK or chicken manure. Silage concentration of lactic acid and ammonia-N from 100% NPK was significantly higher than lactic acid and ammonia-N from 50% NPK treatment. Similarly, use of 100% chicken manure gave significantly higher lactic acid and ammonia-N than fertilizer application of 50% CM. Combination of NPK, organic manure and biofertilizer resulted in increase in silage lactic acid and ammonia-N concentration over control but was similar with 50% NPK+50% CM and 100% NPK application.

Chemical, chicken manure, biofertilizer and combination of these fertilizers effects were not significant for pH value where pH of silage ranged from 4.02 to 4.03. Application of different fertilizer treatments effects were not significant for silage DM which ranged from 32.24% to 32.78%. Furthermore, sole and combination fertilizer treatments effects were not significant for acetic, propionic and butyric acid concentration.

Biological nitrogen fixation (BNF)

There were significant effects of fertilizer treatments on the acetylene reduction assey of corn and soybean roots. Acetylene reduction assey (ARA) of corn roots responded well to treatments with biofertilizer in comparison

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with chemical application (P<0.05). Reducing the level of nitrogen in the fertilizer treatments led to significant increases in acetylene reduction assey. ARA of biofertilizer treatment was generally higher than other sole fertilizer treatments. Among the sole fertilizer treatments, significant difference between 100% NPK, 100% CM and biofertilizer application was recorded. The BF showed higher value of ARA (13.33 nmol/h) than 100% chicken manure (2.61 nmol/h) and 100% NPK (2.42 nmol/h). However, significant difference was not observed among 100% NPK and 100% CM fertilizer (P<0.05) while the control produced significantly higher value of ARA (7.63 nmol/h) than sole chemical and chicken manure fertilizer (Figure 4).

Combined use of biofertilizer with NPK or chicken manure resulted in increasing ARA. Treatments with biofertilizer produced higher rate of ARA but there was no significant difference among biofertilizer treatments. Application of sole BF showed similar ARA as that 50% CM+50% NPK+BF (18.94 nmol/h), 50% CM+BF (10.19 nmol/h) and 50% NPK+BF (9.89 nmol/h) fertilizer (P>0.05).

Acetylene reduction assey of soybean roots responded well to application of biofertilizer in comparison with 100% chicken manure and 100% NPK application (P<0.05). Among the sole fertilizers, significant difference between 100% NPK, 100% CM and biofertilizer application was observed. The biofertilizer application showed higher value of ARA (45.02 nmol/h) in comparison with 100% chicken manure (20.74 nmol/h) and 100% NPK (9.01 nmol/h). However, significant difference was not observed between 100% NPK and 100% CM fertilizer (P<0.05) while the control produced significantly higher value of ARA (28.12 nmol/h) than sole chemical and chicken manure fertilizer. ARA was not significantly different from that of biofertilizer and no fertilizer plots.

Acetylene reduction assey of BF (45.02 nmol/h) was not significantly different from that of 50% CM+50% NPK+BF (34.74 nmol/h), 50% NPK+BF (34.46 nmol/h) and 50% CM+BF (34.22 nmol/h) plots (p<0.05). In this experiment, biofertilizer application (sole or combination with other fertilizer) had significantly affected ARA. Treatments with application of all rate of biofertilizer produced higher value of ARA but there was no significant difference among biofertilizer treatments and control plots.

DISCUSSION

Results showed that application of 100% NPK fertilizer was the best in terms of yield and quality of mixed forage of corn and soybean, which was better than 100% chicken manure application. However, combination of 50% NPK+50% CM gave similar results, which implies that chicken manure can substitute for half of NPK without affecting DM yield of forage. Biofertilizer did not give any benefit on forage yield and quality, alone or in combination with NPK or chicken manure, so application of biofertilizers could not be recommended.

Short plant height was due to depletion of nutrients from control plots over time hence plants showed stunted growth owing to inadequate supply of nutrients. The higher plant height might be attributed to the gradual release of essential nutrient from chemical and chicken manure fertilizer as required by the corn plant. The results of the experiment confirm the findings of Gonzalez et al. (2001) who reported that chemical fertilizer and organic manure which was supplied as essential nutrition at initial establishment stage recorded the best results for the measured parameters such as plant height of the crop. Increased plant height with application of combined fertilizer is attributed to more availability of nitrogen both from urea as well as manure throughout the growing season. These results are in agreement with the findings by Mitchell and Tu (2005) and Saleem (2010) who reported that 50% poultry manure+50% chemical gave the tallest corn plants compared to control plots in the corn-legume cropping systems.

Application of NPK and combined use of organic and chemical fertilizer enhanced corn-soybean forage productivity and yield through increasing LAI and CGR. Corn as a cereal crop responded well to exposure of the leaves to light and uptake of essential nutrients might be attributed to synergic action of fertilizer and organic amendments. These increases in plant growth traits appeared to be more evident when chicken manure was combined with the NPK fertilizer. Corn has a determinate type of growth habit and yield is determined at the early growth stage. Plant availability of micro and macronutrients at the vegetative stage is important and this was provided by the faster release of nutrients from the inorganic fertilizer compared to organic fertilizers. Thus 100% NPK gave a better yield than 100% CM but when the two were mixed, yield was similar to that of 100% NPK.

The finding of this experiment is similar to Khan et al. (2009) who reported that fertilizer application of nitrogen combined with farmyard manure resulted in increased plant height, more LAI and higher and biological yields of corn than the use of organic fertilizer alone. The increased LAI observed with the application of organic and inorganic combination resulted in increased CGR (Naing et al., 2010). The results were in agreement with observation made by Lelei et al. (2009), Saleem (2010), Ibeawuchi et al. (2007) and Khan et al. (2009) who found that higher values of LAI, CGR and higher biological yields of corn was recorded by combined fertilizers than straight fertilizers.

Corn growth with complementary chemical+organic fertilizers and with sole inorganic fertilizer treatment were comparable because nutrients were released early from the inorganic fertilizer and corn, being an aggressive feeder, was able to utilize it for its growth. Although the rate of application of inorganic fertilizer was reduced in the combined use, complementation with nutrients from organic manure made comparable yields as from sole inorganic fertilizer application realizable. Chung et al. (2000) were also in the opinion that organic manures fertilizer application with an adequate amount of inorganic fertilizer gave higher DM yield of corn.

The use of chemical fertilizer has some importance over organic manure because it supplies readily the nutrients to crop which help to boost up the growth and yield (Meng et al., 2005; Manna et al., 2005). The chicken manure is also rich source of nutrients which helps to improve the crop yield and the reduction in yield compared to chemical fertilizer might be due to slow release of the nutrients. The increase in 50% NPK+50% chicken manure application might be due to high level of microbial activity which enhanced organic matter decomposition as well as release of plant available nutrient.

Besides supplying nutrients, organic manures also improve soil structure. Organic manure application enhanced soil organic matter and soil nutrients, which were released slowly and steadily and efficiently utilized during later growth stages of corn. The optimum yield obtained was partly attributable to integration of organic and inorganic fertilizer because nutrients were released from chemical fertilizers and corn was able to utilize it for its growth, supplemented by necessary nutrients released from decomposition of added

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organic manure. These results confirmed studies by other authors e.g. Adeniyan and Ojeniyi (2005); Shata et al. (2007), Sial et al. (2007), Rehman et al. (2008), Law-Ogbomo and Remison (2009). Vanlauwe et al. (2002) reported that integrated use of inorganic and organic nutrient sources result into synergy and improved conservation and synchronization of nutrient release and crop demand, leading to increased fertilizer efficiency and higher yields. Dudal and Roy (1995) indicated that use of organic fertilizer could increase efficiency of inorganic fertilizer.

While some research indicates higher chemical fertilizer may suppress soybean yield (Hiebsch and McCollum, 1987), the positive response of corn to NPK (Jahanzad et al., 2014) compensated for probable yield suppression of soybean, which ultimately resulted in the highest forage DM yield at 100% NPK and the 50% chicken manure+50% NPK treatment.

Chemical fertilizers are rich equally in all three essential nutrients. On the other hand, organic fertilizers may be rich in one of the three nutrients, or may have low levels of all the three nutrients. Chemical fertilizers are always there to provide immediate supply of nutrients to plants if situation demands. In contrast to inorganic fertilizer, one aspect of the organic fertilizers is their slow-release capability. Slow-release means there is less risk of over-fertilization but sometimes this slow-release of organic fertilizers is not able to fulfill to needed supply of the nutrients, whenever required.

Nitrogen is present in manure in a variety of forms, most of which gradually converts to ammonium and nitrate nitrogen. It is the same form ultimately available to plants from chemical fertilizers. Organic fertilizer such as manure adds nutrients to soil, increases soil organic matter, improves soil structure and improves buffering capacity against fluctuations in pH levels.

Sole organic fertilizer or biofertilizer will not meet the nutritional needs of crops because they contain a comparatively less quantity of nutrients compared to chemical fertilizers. Results of this study showed that DM yield from combination of inorganic and organic fertilizer were higher than sole application of organic or biofertilizer. Makinde et al. (2001) have indicated that corn performance from sole chemical fertilizer and a mixture of organic and inorganic fertilizer applications were significantly higher than yields

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from sole organic fertilizer application. Similarly, Murwira and Kirchman (1993) have recorded that the nutrient use efficiency of a crop is increased through an integrated application of mineral fertilizer and organic manure.

Satyanarayana et al. (2002) have observed an optimum grain yield of rice with an application of 10 t ha^{-1} farmyard manure complemented with 120 kg N compared to sole manure and sole inorganic fertilizers. This was attributed to increased nutrient uptake and increased number of tillers and filled grains per panicle. Complementation of chicken manure and NPK fertilizer could therefore be considered as a better option in increasing fertilizer use efficiency and providing a more balanced supply of nutrients (Suge et al., 2011).

In the present study, results showed that biofertilizer application did not benefit the DM yield of forage significantly. Application of biofertilizer did not significantly increase DM yield compared to combination of NPK and chicken manure. There may also be cases where the biofertilizers have insufficient amount of nutrients, which can change the way the plants grow (Lodwig and Poole, 2003).

Biofertilizers are different from chemical fertilizers or organic manure application in their performance on the crop productivity. Biofertilizers have effect on fixation of nitrogen or enhances the availability of nutrient, whereas application of chemical fertilizer directly provide nutrient to the plant at a rapid speed. The nutrient release rate is too slow to meet crop requirements in a short time, hence some nutrient deficiency may occur. Biofertilizers maintain or increase soil microbial complex with slow release of mineral nutrients from organic matter-long term effect and they do not directly supply any nutrients to crops. The major plant nutrients may not exist in biofertilizer in sufficient quantity to sustain maximum crop growth (Chen, 2006). They are comparatively low in nutrient content, so larger volume is needed to provide enough nutrients for crop growth (Chen, 2006; Carvajal-Muñoz and Carmona-Garcia, 2012). There is a gap in biofertilizers use and nutrient requirement of crop, because the field performance of applied biofertilizers depend on fertility status of field soil, type of the crop and method of cultivation etc. Shortages of particular strains of microorganisms or of the best growing medium reduce the availability of some bio fertilizers.

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The quality traits of forage were significantly improved by application of fertilizer. Forage DM yield and protein yield increased with application of 100% NPK, sole chicken manure and combined NPK with CM, compared to sole biofertilizer and unfertilized treatments. These results were in agreement with the findings by Abbasi et al. (2012) who recorded significant increase corn-amaranth forage yield and concentration of CP with fertilizer application compared to without fertilizer treatments.

Likewise, CP content significantly increased as chemical fertilizer and combination of inorganic with CM fertilizer. These results coincided to with findings of some workers (Soto et al., 2004). Nitrogen contributes greatly to synthesis of amino acids and ultimately proteins, the higher nitrogen available to the crops; the higher protein can be synthesized. High levels of CP as a result of NPK application which leads to increased protein synthesis have been reported in sorghum-lima bean forage (Zandvakili et al., 2012) and forage amaranth production (Abbasi et al., 2012).

Application of 100% NPK fertilizer increased CP concentration but it also increased NDF and ADF concentration. However, when combined with chicken manure it resulted in lower NDF and ADF concentration. These results confirm earlier reports by Abbasi et al. (2012) who reported increased NDF content in forage amaranth with chemical fertilizer application. Some researchers reported meaningful results indicating that organic fertilization decreased ADF concentration in corn production (Keskin et al., 2005). Organic manure fertilizer significantly reduced the NDF concentration. Application of 100% of CM and combined CM with NPK and BF produced lower NDF and ADF concentration than other treatments.

Fertilizer application influenced some fermentation characteristics of corn-soybean silage. Lactic acid responded differently to different fertilizer application and was significantly higher than the no fertilizer plots. Generally, presence of high levels of lactic acid suggests an efficient fermentation and minimal DM loss of silage (Seglar, 2003). According to Seglar (2003), quality silage should contain <1 g kg^{-1} butyric acid, and elevated levels may cause silage deterioration due to secondary fermentation while acetic, propionic and butyric acid content were not influenced by fertilizer application.

Higher concentrations of ammonia–N results from hydrolysis of protein and affects silage quality adversely (Kung and Shaver, 2001). These criteria characterize the silage produced in intercropping with application of NPK and CM and combined use of these fertilizers in this study as being good silage.

A chemical fertilizer is defined as any inorganic material of wholly or partially synthetic origin that is added to the soil to sustain plant growth. Many artificial fertilizers contain acids, such as sulfuric acid and hydrochloric acid, which tend to increase the acidity of the soil, reduce the soil's beneficial organism population and interfere with plant growth. In contrast, organic fertilizers support the growth of nitrogen-fixing bacteria. Generally, healthy soil contains enough nitrogen-fixing bacteria to fix sufficient atmospheric nitrogen to supply the needs of growing plants. However, continued use of chemical fertilizer may destroy these nitrogen-fixing bacteria.

In summary, no fertilizer treatment, biofertilizer and combination of biofertilizer with NPK or CM treatments tended to have higher rates of acetylene reduction assey compared to 100% NPK and 100% chicken manure fertilizers. There did not appear to be much difference among the biofertilizer and combination and control in term of ARA. Treatments that supply high level of nitrogen had lower value of ARA and maximum ARA was recorded in treatments with low level of nitrogen.

CONCLUSION

Among fertilizer treatments in this experiment, conventional fertilizer 100% NPK, and 50% CM+50% NPK effects were similar and significantly increased corn-soybean DM yield, improved plant growth characteristics, forage quality, CP concentration, protein yield and quality of corn-soybean silage. Chicken manure (CM) has come to serve as one of the most important manure as it consists of higher nitrogen value. The chicken manure is locally available hence smallholders farmers can easily afford it as compared to expensive chemical fertilizer, also manure is environmental friendly. Complementary application of chemical fertilizers and organic manure can be a fertilizer management strategy in corn+legume intercropping systems. Biofertilizer application (sole or combination with other fertilizer) had no significant benefit on forage DM yield and nutritive quality. Integrated use of chicken manure and chemical fertilizers may be practiced to achieve proper productivity and quality of corn-soybean silage.

Increasing the level of nitrogen (100% NPK or 100% chicken manure) led to significant decrease in acetylene reduction assey. There is no simple and easy approach to increase BNF in grain legumes grown as part of a cropping system, under realistic farm field conditions. Numerous (micro)-climatic variables, soil physical properties, agronomic management, host-rhiozobia combination and socioeconomic aspects play an important role in controlling BNF. Management practices that increase nitrogen demand by the host plant is a promising avenue to increase N fixation in grain legumes in a cropping system.

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Figure 1(on next page)

Diagram

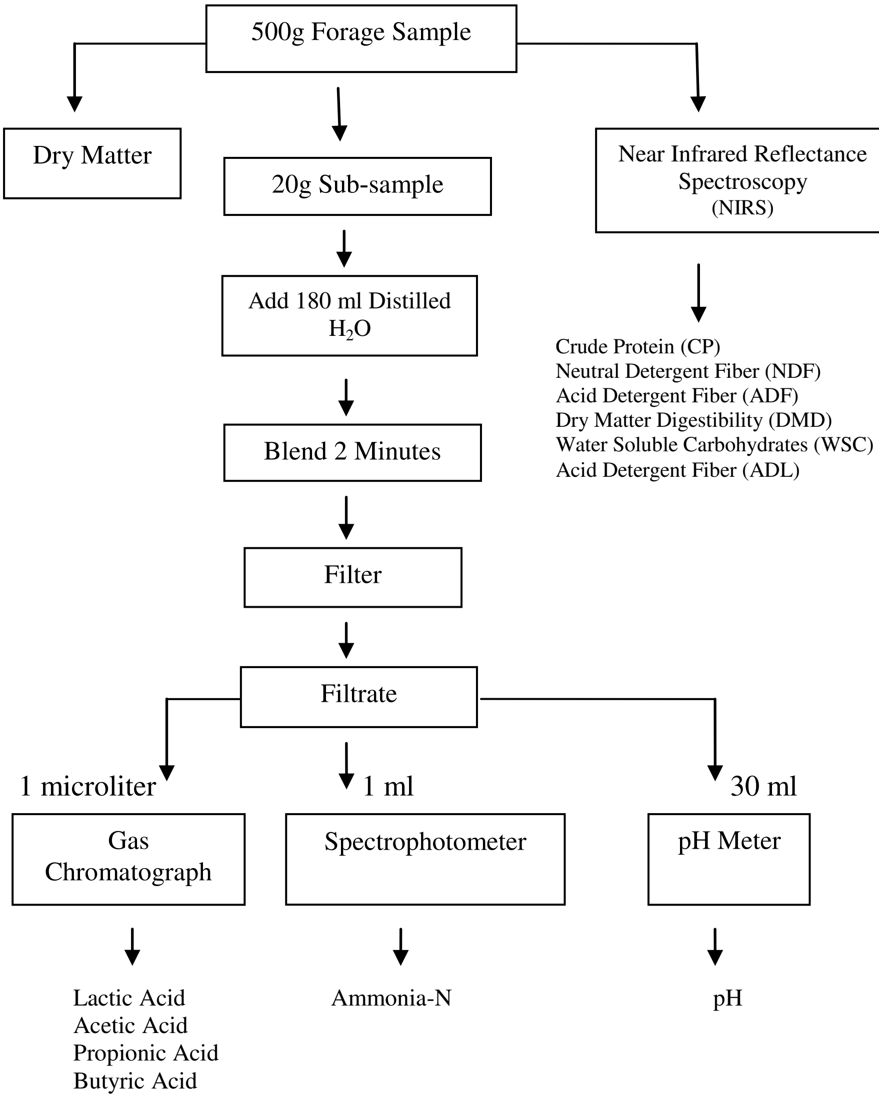

Figure 1: Fermentation analysis flow diagram

Figure 2(on next page)

Table

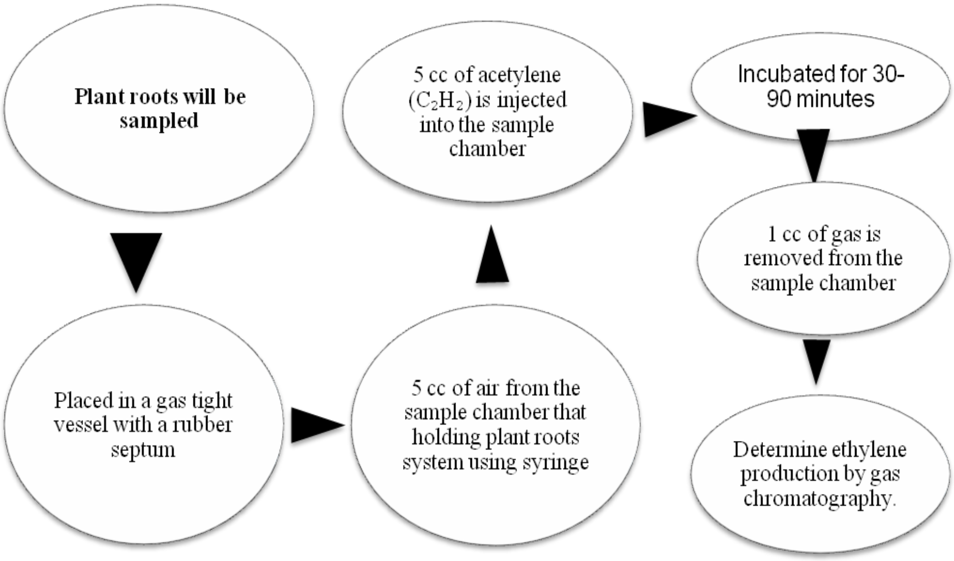

Figure 2: Acetylene reduction method to measure nitrogenase enzyme activity

Figure 3(on next page)

Forage dry matter (DM) yield

Forage dry matter (DM) yield of intercropped corn and soybean as influenced by integrated nutrient management

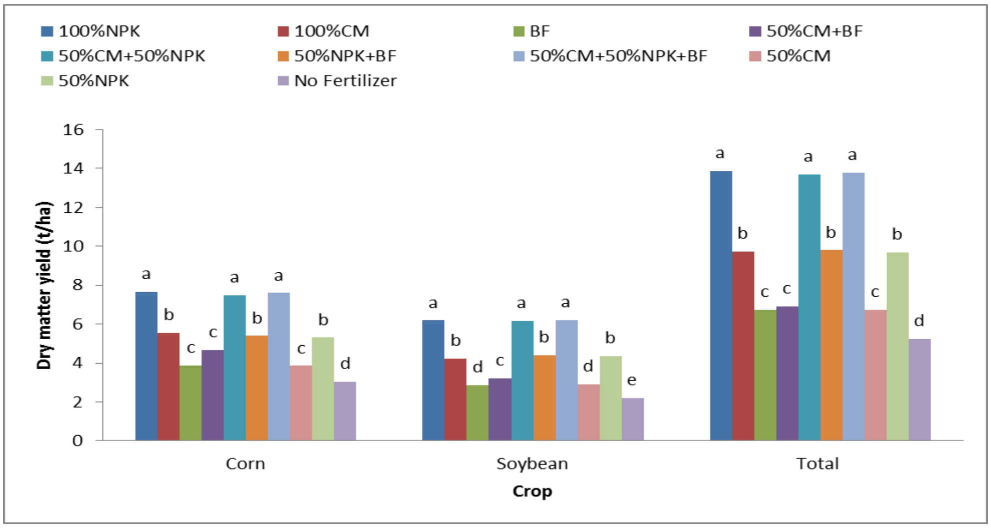

Figure 3: Forage dry matter (DM) yield of intercropped corn and soybean as influenced by integrated nutrient management

Figure 4(on next page)

Acetylene reduction assey (ARA)

Effect of chemical, organic and biofertilizer on acetylene reduction assay (ARA) of corn and soybean roots

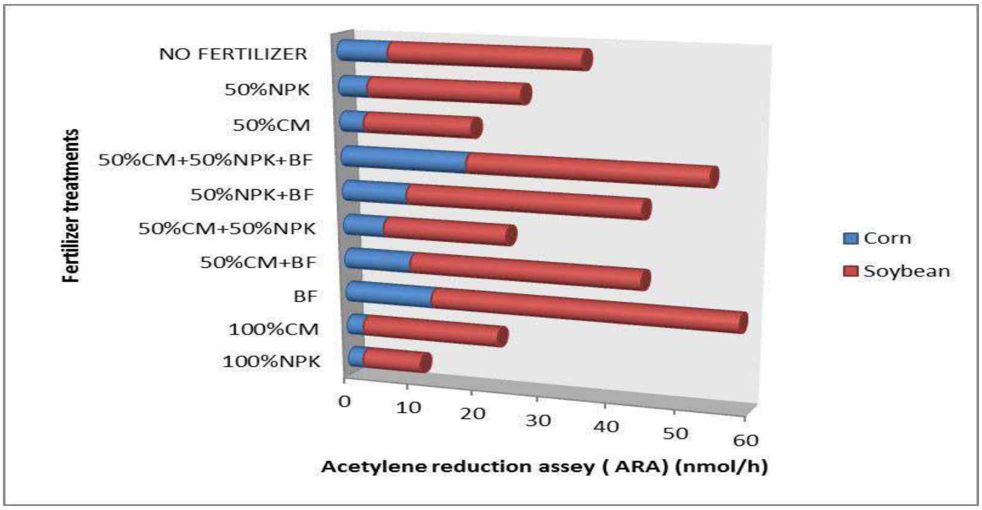

Figure 4: Effect of chemical, organic and biofertilizer on acetylene reduction assey (ARA) of corn and soybean roots

Table 1 (on next page)

Treatments

List of treatments

Table 1: List of treatments

Treatment	Description
T1	100% NPK or conventional fertilizer
T2	100% chicken manure (CM)
T3	Biofertilizer
T4	50% chicken manure + biofertilizer
T5	50% NPK + 50% chicken manure
T6	50% NPK+ biofertilizer
T7	50% NPK + 50% chicken manure + biofertilizer
T8	50% chicken manure
T9	50% NPK
T10	No fertilizer

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Table 2 (on next page)

Plant growth parameters

Plant growth parameters of intercropped corn and soybean as influenced by integrated nutrient management

Table 2: Plant growth parameters of intercropped corn and soybean as influenced by integrated nutrient management

Treatment	**Plant height** corn (cm)	**Plant height** soybean (cm)	**Crop growth rate** ($g/m^2/day$) corn	**Crop growth rate** ($g/m^2/day$) soybean	**Leaf area index** corn	**Leaf area index** soybean
100%NPK	197.00a	109.25ab	29.34a	9.55a	2.56a	3.17a
100%CM	184.00b	99.00c	26.37b	8.48b	2.39b	3.07b
BF	152.50c	76.50d	22.51c	7.75c	2.15c	2.78c
50%CM+BF	152.00c	77.75d	22.40c	7.72c	2.12c	2.75c
50%CM+50%NPK	195.25a	112.75a	28.98a	9.39a	2.52a	3.20a
50%NPK+BF	185.00b	100.00c	26.98b	8.63b	2.37b	3.05b
50%CM+50%NPK+BF	195.00a	112.00a	29.23a	9.61a	2.54a	3.20a
50%CM	153.00c	78.00d	22.48c	7.78c	2.12c	2.78c
50%NPK	186.25b	99.75c	26.35b	8.55b	2.34b	3.02b
No Fertilizer	127.00d	64.00e	17.80d	7.26d	1.84d	2.54d
LSD(0.05)	3.49	2.20	1.002	0.40	0.04	0.05
P Value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Mean values followed by the same letter in the same column are not significantly different at $P<0.05$, based on least significant difference test (LSD).

Table 3 (on next page)

Forage nutritive quality

Forage nutritive quality of intercropped corn and soybean as influenced by integrated nutrient management

Table 3: Forage nutritive quality of intercropped corn and soybean as influenced by integrated nutrient management

Treatment	**Crude protein** (%)	**Neutral detergent fiber** (%)	**Acid detergent fiber** (%)	**Dry matter diges tibility** (%)	**Water soluble carbohy drates** (%)	**Acid detergent Lignin** (%)	**Protein yield** (Kg/ha)
100%NPK	14.48a	57.34a	35.68a	66.31	22.02	3.67	2009.98a
100%CM	14.50a	49.22b	26.66b	65.69	22.16	3.69	1408.95b
BF	13.02c	58.13a	36.09a	66.53	22.36	3.74	894.25d
50%CM+BF	13.38b	58.00a	36.17a	66.98	22.17	3.75	923.74d
50%CM+50%NPK	14.55a	49.67b	26.79b	66.73	22.15	3.62	1991.95a
50%NPK+BF	13.45b	58.52a	36.12a	65.87	22.47	3.77	1307.87c
50%CM+50%NPK+BF	14.56a	49.46b	26.74b	66.82	22.73	3.70	2005.75a
50%CM	13.28b	57.39a	35.23a	66.95	22.43	3.80	896.03d
50%NPK	13.33b	57.73a	35.30a	66.92	22.70	3.74	1288.39c
No Fertilizer	12.28d	57.93a	36.48a	66.36	22.21	3.76	642.62e
LSD(0.05)	0.33	1.64	1.84	ns	ns	ns	69.19
P Value	<.0001	<.0001	<.0001	0.3899	0.9919	0.9905	<.0001

Mean values followed by the same letter in the same column are not significantly different at P<0.05, based on least significant difference test (LSD).

Table 4(on next page)

Silage volatile fatty acids (VFA)

Silage volatile fatty acids (VFA) of intercropped corn and soybean as influenced by integrated nutrient management

Table 4: Silage volatile fatty acids (VFA) of intercropped corn and soybean as influenced by integrated nutrient management

Treatment	**pH**	**DM range** (%)	**Lactic acid** (% of DM)	**Acetic acid** (% of DM)	**Propionic acid** (% of DM)	**Butyric acid** (% of DM)	**Ammonia N** (% of DM)
100%NPK	4.02	32.61	4.04a	1.62	0.16	0.03	2.06a
100%CM	4.03	32.24	4.02ab	1.63	0.16	0.03	2.07a
BF	4.02	32.52	3.91d	1.63	0.15	0.04	1.84b
50%CM+BF	4.03	32.49	3.92cd	1.63	0.16	0.03	1.83b
50%CM+50%NPK	4.03	32.77	4.01abc	1.62	0.16	0.03	2.08a
50%NPK+BF	4.03	32.33	3.93bcd	1.64	0.15	0.03	1.83b
50%CM+50%NPK+BF	4.02	32.78	4.03a	1.63	0.14	0.03	2.04a
50%CM	4.02	32.39	3.92cd	1.63	0.14	0.03	1.82b
50%NPK	4.02	32.25	3.92cd	1.62	0.15	0.03	1.83b
No Fertilizer	4.03	32.44	3.89d	1.64	0.15	0.04	1.81b
LSD(0.05)	ns	ns	0.10	ns	ns	ns	0.19
P Value	0.9999	0.6425	0.0120	0.9992	0.9246	0.9532	0.0065

Mean values followed by the same letter in the same column are not significantly different at P<0.05, based on least significant difference test (LSD).