

FERMENTATION CHARACTERISTICS AND NUTRITIVE VALUE OF CORN SILAGE INTERCROPPED WITH SOYBEAN UNDER DIFFERENT CROP COMBINATION RATIOS

A. Baghdadi¹, R. A. Halim^{1*}, O. Radziah², M.Y. Martini¹ and M. Ebrahimi³

¹ Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia

² Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia

³ Faculty of Veterinary Medicine, Universiti Putra Malaysia

* Corresponding Author E- mail: ridzwan@upm.edu.my

ABSTRACT

Corn silage is an important feed for intensive ruminant production. Combining corn with legumes for silage is a feasible strategy to improve crude protein (CP) concentration in corn silage. This study was conducted to determine silage nutritive quality and fermentation profiles of corn grown in mixture with soybean at different crop combination ratio. In this experiment, corn-soybean combinations of 75:25, 50:50 and 25:75 in addition to monocrops of corn and soybean were evaluated. The crop combination ratio had significant effects on nutritive quality and fermentation characteristics of silage. Silage quality in terms of CP (75:25 ratio 12.23%, 50:50 ratio 12.88% and 25:75 ratio 13.65%) was improved by intercropping compared with corn sole crop (9.91% CP). Increase ratio of soybean resulted in an increase in lactic acid and pH of the mixed silage. Sole soybean gave significantly higher lactic acid (3.57%) and pH of silage (4.33) compared to all other treatments but dry matter concentration was significantly higher in corn monocrop silage (34.34%) than other treatments. The propionic acid and butyric acid content of soybean monocrop and intercrops silage were in all cases higher than sole corn silage.

Keywords: Silage fermentation, volatile fatty acids, nutritive quality, proportion, corn-soybean intercropping.

INTRODUCTION

Intercropping is widely practiced in many regions of the world for the production of food and feed crops. Improvement of forage quality through the corresponding effects of two or more species grown together on the same area of land is one of the main advantages of intercropping (Bingol *et al.*, 2007; Lithourgidis *et al.*, 2007; Ross *et al.*, 2004).

Intercropping of corn and legume is considered best for subsistence food production system (Tsubo *et al.*, 2005; Egbe and Adeyemo, 2006). Successful intercropping of corn and soybean has been reported since soybean is often used to increase the typically low protein content of corn silage. Substantial information has been obtained on the corn-soybean model (Geren *et al.*, 2008).

Previous studies have suggested that intercropping grasses with legumes may be a feasible option to increase protein content and quality of silage and increase the viability of dairy farming system (Titterton and Maasdorp, 1997; Sadeghpour *et al.*, 2013 a, b). Kennelly and Weinberg, 2003 reported that high quality silage could be made by mixing legumes and cereal crops during ensiling of forage. Grain and forage cultivars of soybean are considered as promising legumes for silage production (Lima-Orozco *et al.*, 2012). In addition, forage soybean contains higher levels of protein

than many other types of forage and possesses N fixation capability; however, some studies suggest that soybean has poor fermentation quality when ensiled as a monoculture (McDonald *et al.*, 1991).

Corn is a high-energy, high yield forage crop (Jorge and Joseph, 1999). Similarly, Emine *et al.* (2010) mentioned that the characteristic of good fodder corn is high forage yield and high digestibility.

The crop combination ratio can influence corn-soybean forage yield and silage quality. In combination ratio of 50:50 of corn and 15 different legumes CP concentration was increased compared to corn monocrop but digestibility decreased (Titterton and Maasdorp, 1997). Corn and cowpea seed mixed in various combinations affected the production of crude protein, where, an increased ratio of cowpea in seed mixture increased the CP concentration. The sole cowpea produced more CP (18.10%) and corn monocrop produced lower content of CP (8.5%). The crop combination of 75:25 corn and cowpea produced more CP (10.45%) than sole corn crop (Ibrahim *et al.*, 2006).

It is known that legumes have higher buffering capacities than grasses, which tend to extend fermentation, slow the drop of pH, and increase proteolysis (McDonald *et al.*, 1991; Albrecht and Muck, 1991). Moreover, it is also well documented that not all legumes ferment equally when in monoculture (Mustafa

and Seguin, 2003; Albrecht and Beauchemin, 2003) or in mixture (Dawo *et al.*, 2007).

It is well documented that corn is able to provide high dry matter yield and low protein content forage. However, livestock need a good protein source for their growth and milk production. Protein is also essential for rumen bacteria, which digest the feed for ruminant animals (Ghanbari-Bonjar, 2000). Lawes and Jones, 1971 stated that corn-hay is usually lower than that which is needed to meet rational production levels for many groups of livestock due to their low protein content. Therefore, it seems essential to support livestock with protein increments when silage quality is low.

The silage fermentation qualities have been described by research laboratories in recent years. Fermentation characteristics reported include dry matter, pH, ammonia, acetic acid, lactic acid, propionic acid, and butyric acid (Kung and Shaver, 2001; Ward, 2000). In recent years, more attention has been paid to the pH of a fermented feed as an indication of the quality of the fermentation (Ward, 2000). Lactic acid should be the primary acid in good silage. This acid is thought to be stronger than the other acids in silage (acetic, propionic, and butyric), and therefore is usually responsible for most of the drop in silage pH. Additionally, fermentations that produce lactic acid result in the lowest losses of DM and energy from the crop during storage (Kung and Shaver, 2001). The main goal of making silage is to maximize the preservation of the original nutrients in the forage crop for feeding later. However, fermentation in the silo is a much-uncontrolled process usually leading to less than optimal preservation of nutrients (Kung, 2001).

Therefore, the objective of this study was to evaluate nutritive quality and fermentation profiles silage corn intercropped with soybean under different crop combination ratios.

MATERIALS AND METHODS

Experimental location, climate and soil: An experiment was conducted at research field, Universiti Putra Malaysia (UPM) (latitude 3: 02' N longitude of 101: 42' E and altitude 31m above sea level). Mean annual minimum and maximum temperatures was 24.5°C and 32.2°C, respectively, while the mean relative humidity was 78.9%. Total annual rainfall in the year 2013 was approximately 1623.5 mm.

A composite soil sample was collected at random in the entire plot before the experiment to determine the physical and chemical characteristics. Soil texture was a sandy loam type. Textural analysis showed that the soil contains 18.77% clay, 18.41% silt and 62.61% sand with an organic carbon 1.55%, pH of 6.18 at 0-30 cm depth. Details of soil physical and chemical properties of the experimental site are given in Table 1.

Table 1. Soil physical and chemical properties of the experimental site (0-30 cm depth).

Soil characteristics	Values
Organic carbon (%)	1.55
Clay (%)	18.77
Silt (%)	18.41
Sand (%)	62.61
Texture	Sandy loam
pH	6.18
EC (ds/m)	3.16
K (ppm)	19.7
N (%)	0.11
P (ppm)	18.7

Agronomic practices: The previous crop on this field was corn. The land was ploughed once with mould board plough, harrowed and rotovated twice to bring the soil to fine tilth. The seed moisture and germination percentage were 15 and 95%, respectively. Corn and soybean intercrops were planted at the same time. The sowing date of the field experiment was on November 2013. Two seeds were hand planted per point and plants were thinned to the target population just prior to the six-leaf stage.

Based on soil analysis, the required levels of N, P and K to support the yield goals were 130 kg ha⁻¹ N, 130 kg ha⁻¹ P and 80 kg ha⁻¹ K in the form of urea, triple super phosphate (TSP) and Muriate of potash (MOP). The whole of phosphorus and potassium and one-third of N fertilizer were applied at the sowing time. The rest of N fertilizer was applied at 8-leaf stage of corn. Each plot was covered with plastic sheet mulch in order to control the growth of weeds within the plots. Irrigation water was adequately applied using a sprinkler system. All agronomic practices except those under study were kept uniform for all treatments.

Experiment design and treatments: The experiment was established in a RCBD with four replications. Corn (variety 926) was intercropped with soybean (*Glycine max*). The treatments consisted of five-crop combination ratio of corn and soybean (100:0, 75:25, 50:50, 25:75, and 0:100). The size of each plot was 10.0 m long and 5.0 m wide. A buffer zone of 2.0 m spacing was provided between plots. The intercrop composition was based on replacement design. Each experimental unit was composed of eight crop rows, each 10 m long. Within rows, spacing of corn was 20 cm while for legumes it was 10 cm with inter row spacing of 60 cm. One plot comprised of corn monocrop and one plot comprised of soybean monocrop

Silage preparation: Harvested forages from each plot were chopped with a forage chopper. The forage was then ensiled in plastic drums of either 20-25 liters capacity

after compacting them with an Otosilager® (Forage compacting machine). Silos were stored in a laboratory with temperatures ranging from 25 to 30 °C. After 10 weeks of ensiling, silos were opened and top 10 cm of the corn-legumes and corn silage was discarded and 20 g of representative silage were mixed with 180 g sterile water in a laboratory blender (Waring, New Hartford, Conn, USA) for 2 minutes. The extract was filtered through four layers of gauze and no.1 filter paper (Whatman, Inc., Clifton, NJ). The filtrate extract was used for measuring pH, ammonia nitrogen, lactic acid and volatile fatty acids (VFA). The pH was determined using a pH electrode (Mettler-Toledo Ltd., England) immediately after the sample preparation.

Nutritive value in silage: After 10 weeks of ensiling, silos were opened, the top 10 cm of the corn-legumes and corn silage was discarded, and 500 g sub-sample of each ensiled samples weighed and were dried for 3-7 days in a 70 °C forced-air oven to constant moisture to determine silage nutritive quality characteristics including crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), dry matter digestibility (DMD), water soluble carbohydrates (WSC) and acid detergent fiber (ADL). Near infrared reflectance spectroscopy (NIRS) technology, using a global calibration equation was used to estimate the nutritive quality of silage (Jafari *et al.*, 2003).

Determination of volatile fatty acids in silage: Volatile fatty acids were determined using gas-liquid

chromatography with Quadrex 007 Series (Quadrex Corporation, New Haven, CT 06525 USA) bonded phase fused silica capillary column (15m, 0.32mm ID, 0.25 µm film thickness) in an Agilent 7890A gas-liquid chromatography (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame ionization detector (FID). The injector/detector temperature was programmed at 220/230 °C, respectively. The column temperature was set in the range of 70 °C - 150 °C with temperature programming at the rate of 7 °C/min increments to facilitate optimal separation. Peaks were identified by comparison with authentic standards of lactic, acetic, propionic, butyric acids (Sigma, St. Louis, Mo., USA). Methyl n-valeric acid and fumaric acid as internal standard used for VFA and lactic acid determination, respectively. Fermentation analysis of silage is shown in Figure 1.

Determination of ammonia-N in silage: Concentrations of ammonia-N were determined using the colorimetric method described by Solorzano (1969). A standard curve was made to determine whether a linear relationship existed between the varying concentrations of an ammonium sulfate standard solution and the intensity of color produced. The intensity of color thus developed was measured at a wavelength of 420 nm using a spectrophotometer (Secomam, Domont, France) within 5–10 min after setting it at 0 absorbance with the blank (Solorzano, 1969).

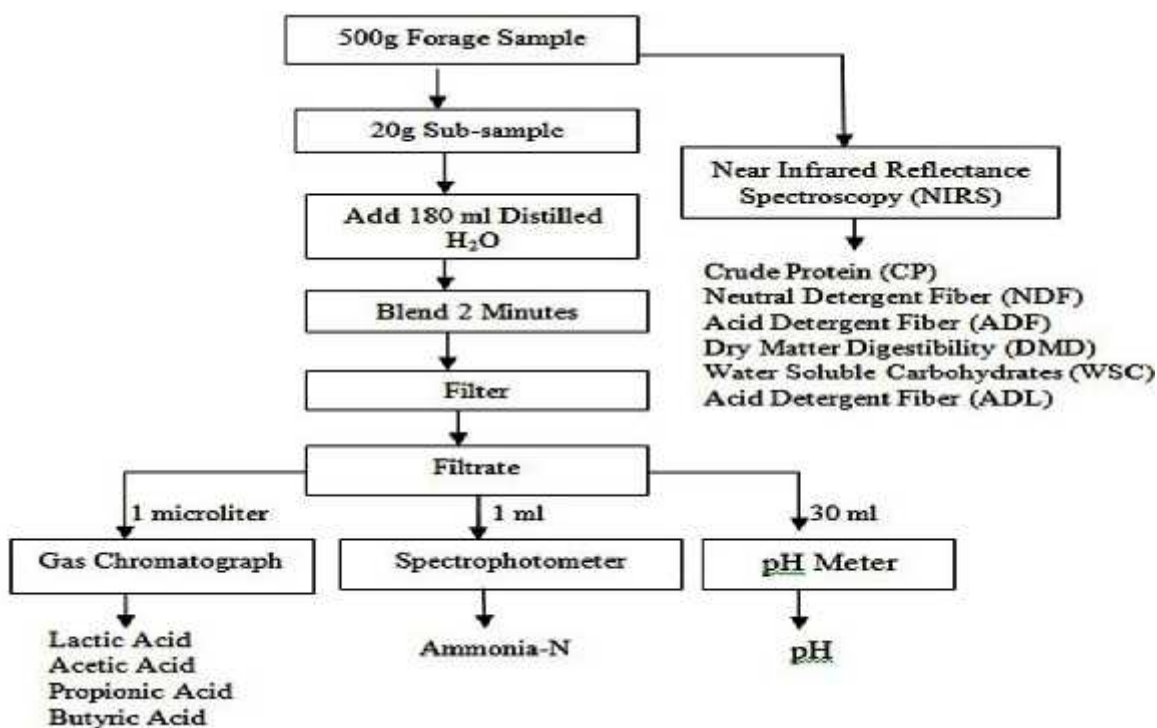


Figure 1. Fermentation analysis flow diagram

Statistical analysis: All data were analyzed with the analysis of variance (ANOVA). The Least Significant Difference (LSD) test was used to compare treatment means at the 0.01 and 0.05% probability levels. The Mixed Linear Model in SAS Statistical Software Package (Version 9.1) was used to perform an analysis of variance appropriate for a RCBD.

RESULTS

Nutritive quality in silage: Intercropping had significant effect on CP, NDF, DMD, WSC and ADL concentration of silage of sole crops and intercropped treatments. Monocultures of corn and soybean and intercropping treatments with various crop combination ratio, were significantly different in terms of silage nutritive parameters (Table 2).

The CP content was significantly affected by crop combination ratio. The CP content declined with decrease in proportion of soybean from 16.24% to 9.91%

The NDF was significantly affected by crop combination ratio (Table 2). The highest NDF content

was for the corn monocrop (51.57%), 75:25 (51.07%) and 50:50 (48.34%) corn-soybean ratio.

Corn-soybean combination had a significant effect on ADF content of silage. Corn sole crop and 75:25 corn-soybean ratios produced significantly higher concentration of ADF (32.82 and 32.58%, respectively) than the other treatments.

Combination ratio of corn-soybean had significant effect on dry matter digestibility (DMD) of forage. Combination of corn-soybean in all ratios gave significantly higher DMD than corn monocrop.

Crop combination ratio had a significant effect on water soluble carbohydrate (WSC) content of forage. Increase ratio of soybean resulted in reduction of WSC in the mixed forage. The WSC content was similar for sole crop corn up to 50:50 corn-soybean combinations.

Crop combination ratio had a significant effect on ADL concentration. The ADL content increased when soybean ratio increased in mixed forage. The ADL content was similar for sole crop corn up to 50:50 corn-soybean combinations.

Table 2. Silage nutritive value quality of sole and intercropped corn and soybean as influenced by crop combination ratio

Treat ment	Crude Protein (%)	Neutral Detergent Fiber (%)	Acid Detergent Fiber (%)	Dry Matter Digestibility (%)	Water Soluble Carbohydrates (%)	Acid Detergent Lignin (%)
100 : 0	9.91e	51.57a	32.82a	58.24b	23.52a	3.15b
75 : 25	12.23d	51.07a	32.58a	58.22b	23.29a	3.19b
50 : 50	12.88c	48.43ab	30.05b	63.38a	23.11a	3.35b
25 : 75	13.65b	47.31b	29.63b	63.07a	21.09b	4.06a
0 : 100	16.24a	47.03b	29.94b	63.62a	20.94b	4.38a
P value	<.0001	0.0317	0.0114	0.0214	0.0261	<.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. Silage volatile fatty acids (VFA) of sole and intercropped corn and soybean as influenced by crop combination ratio

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 : 0	3.94c	34.24a	3.57d	1.46c	0.13e	0.03d	1.83e
75 : 25	4.01c	33.71ab	3.78c	1.47c	0.17d	0.05cd	1.54d
50 : 50	4.02c	32.98b	3.92c	1.52c	0.21c	0.06bc	1.95c
25 : 75	4.16b	31.74c	4.08b	1.82b	0.27b	0.08b	2.75b
0 : 100	4.33a	30.29d	4.46a	2.11a	0.36a	0.11a	3.51a
P value	0.0001	<.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).

Volatile fatty acids in silage: Crop combination had significant effects on all the silage fermentation characteristics. Increase ratio of soybean resulted in an increase in pH of the mixed silage. Monocropped corn (3.94), 75:25 (4.01) and 50:50 (4.02) combination ratio provided most favorable pH level, but there was no significant difference between these treatments. Sole soybean gave significantly higher pH of silage (4.33) compared to all other treatments. Dry matter concentration was significantly higher in corn monocrop silage (34.34%) than 75:25 (33.71%), 50:50 (32.98%), 27:75 (31.74%) corn-soybean combination ratio while, soybean monoculture produced the lowest DM (30.29%) (Table 3).

Lactic acid content was highest in sole soybean silage (4.46% of DM) than all other treatments. Lactic acid increased with increasing proportion of soybean in the mixture and the lowest lactic acid concentration was found in the sole corn silage (3.57%).

The acetic acid content increased when soybean ratio increased in mixed silage up to sole soybean silage (2.11%). The acetic acid content was similar for sole crop corn (1.46%) up to 75:25 corn-soybean combinations.

The propionic acid and butyric acid content of soybean monocrop and intercrops silage were in all cases higher than sole corn silage. The highest content of propionic acid was obtained from soybean monoculture (0.36%) than other ratios. Similarly, butyric acid content was highest in soybean monoculture (0.11%) and butyric acid content was lowest in corn monoculture (0.03%).

The ammonia-N level increased with increasing proportion of soybean in the silage. The highest content of ammonia-N was recorded with sole soybean silage (3.51%).

DISCUSSION

Crude protein concentration increased in the mixture when corn proportion in the mixture decreased by 50%. Results in the present study were in agreement with other studies where legumes also increased CP concentration when in a mixture with corn. Liu *et al.*, 2006 reported that intercropping system increased CP concentration by 30.8% and 99.4% as compared with sole corn crop. Likewise, Putnam *et al.* (1986) found 11-51% increases in CP concentrations of corn-soybean intercrops compared with a corn sole crop. Fujita *et al.* (1992) reported that protein content was increased with the different intercropping combination.

Other important attributes for forage quality are the NDF and ADF concentrations (Anil *et al.*, 2000; Bingol *et al.*, 2007; Contreras-Govea *et al.*, 2006; Lithourgidis *et al.*, 2006; Lithourgidis *et al.*, 2007). The NDF content is indispensable in ration formulation as it reflects the amount of forage that can be consumed by animals (Bingol *et al.*, 2007; Lithourgidis *et al.*, 2006;

Van Soet, 1994). Forage quality in terms of ADF and NDF concentration of forage was improved by intercropping as compared to sole corn crop.

The quality parameters of forage as CP percentage increased while NDF, ADF percentage decreased by growing corn in mixture with soybean. These results were in agreement with Buxton (1996) and Caballero *et al.* (1995), who stated that NDF was lower and CP contents was higher in legumes compared to cereals.

The ensiling of forage legumes often results in rapid and extensive degradation of proteins before and during fermentation (Ibrahim *et al.*, 2006). Legumes such as soybean and alfalfa have high protein concentration, low levels of water-soluble carbohydrate and high buffering capacity, making them susceptible to extensive proteolysis during silage fermentation (Contreras-Govea *et al.*, 2006).

Difference in WSC content between cereal and legumes, and high WSC in corn will contribute to a more rapid drop in sole corn and mixture silage pH. Probably the greater buffering capacity of legume (soybean) compared to cereal (corn) resulted in longer fermentation and thus a greater utilization of WSC concentration in comparison with sole corn crop. These results agreed with those of Jahanzad *et al.* (2014) who found that higher pH and lower WSC concentration in sole soybean crop in millet-soybean intercrops research.

There have been other reports from conducted studies in which WSC concentration decreased when the ratio of the legume increased in the mixture (Contreras-Govea *et al.*, 2006). Likewise, in the mixtures of winter wheat with kura clover, a higher ratio of kura clover in the mixture resulted in higher utilization of WSC (64–88%) than in mixtures with a low proportion of kura clover (44–75%) (Contreras-Govea *et al.*, 2006). These results were in contrast to those reported by Stoltz *et al.* (2013) who found a higher concentration of butyric acid in corn monoculture compared with corn-faba bean. Some studies suggest that acetic acid concentrations above 6% of DM will depress animal intake of feed (Buchanan-Smith, 1990). None of the silages in this study reached this value.

The pH is one of the most important factors in assessing silage fermentation, because generally the lower the pH, the better fermented is the silage. In this study, pH increased slightly as corn was substituted with soybean in intercropping ratios. Intercropping ratios showed an increase in pH on average compared with corn monoculture, and the highest pH was with silage produced in soybean monoculture. This increase in pH could be explained by the higher buffering capacity of legumes. The pH of an ensiled sample is a measure of its acidity, but is also affected by the buffering capacity of the crop. Two samples may have the same pH, but different concentrations of acids. In general, legume

silages have a higher pH than corn or other grass silages and take longer to ensile because of their higher buffering capacity (Kung and Shaver, 2001). The pH values had small difference among intercropping combination ratios all combination ratio treatments gave a pH range ideal for silage (Cherney *et al.*, 2007). In addition to the buffering capacity of legumes, the lower pH of silage from corn-soybean mixture silage could also be due the higher WSC content of the corn, which may have resulted in faster rates of fermentation.

Ammonia-N in silage reflects the degree of protein degradation, and extensive proteolysis adversely affects the utilization of N by ruminants (Wilkinson, 2005). Ammonia-N can be high in forages that are high in CP concentration such as legumes (Nkosi *et al.*, 2012). Corn-soybean silage had ammonia-N concentrations that were lower than sole soybean value, which is indicative of well-preserved silage. It is generally accepted that in cereal-legume intercropping, total DM decreases as legume percentage increases in the mixture. In this study, corn monoculture produced the highest DM (34.24%). The effect of intercropping ratios on DM content was minimal and intercropping ratios had DM concentrations within the range for good fermentation (Muck *et al.*, 2003). Similar reductions in DM concentration of mixtures compared to corn monoculture also were reported in other studies (Titterton and Maasdorp, 1997; Anil *et al.*, 2000; Zandvakili *et al.*, 2013; Jahanzad *et al.*, 2014).

Conclusion: Intercropping of corn-soybean under different combination ratio enhanced the silage quality in terms of nutritive value and silage fermentation as compared to the sole cropping of corn. The best ratio of corn-soybean to obtain high quality silage is 50:50. pH increased slightly as corn was substituted with soybean in intercropping ratios. Corn-soybean silage had ammonia-N concentrations that were lower than sole soybean value, which is indicative of well-preserved silage. It is generally accepted that in cereal-legume intercropping, total DM decreases as legume percentage increases in the mixture.

Acknowledgements: This research was supported by the Ministry of Education Malaysia and Universiti Putra Malaysia, as part of Project no. ERGS/1-2013/5527160.

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