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# Prediction of nutritional values of yellow and white corn hybrids silage from chemical composition and *in vitro* gas production technique

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**Abstract:** Single crossings corn hybrids SC 162 (yellow) and SC 10 (white) were cultivated at a rate of 71,400 plants per hectare using 35 kg of grains during the summer season in the north Delta, Kafr El-Sheikh Government, Egypt. The yield of the corn crop and plant parts (ears, stalks, and leaves) were estimated for each subplot and calculated per hectare. Whole corn plants were harvested after 90 days of planting at the dough stage of maturity, chopped to maximum length of 1-1.5 cm, and ensiled in plastic bags for 35 days. Representative samples of corn silage were analyzed for composition, fiber fractions, vitamins, and silage quality and used in gas and methane production to calculate gas parameters. Results revealed that almost all of the parameters analyzed remained nearly similar for yellow and white corn hybrid silage without significant differences. Whereas the contents of β-carotene and vitamin-A were significantly higher ($p < 0.05$) for yellow compared to white corn hybrid silage. We can predict the nutritional values of corn hybrids silage in terms of total digestible nutrients (TDN), digestible crude protein (DCP), gross energy (GE) and digestible energy (DE) from chemical composition. Also, we can predict the nutritional values in terms of feed intake, dry and organic matters digestibility, short chain fatty acids and microbial production and energy content from the *in vitro* gas Production. So, it can be predicted the nutritional values of corn silage easily and quickly in laboratory using the methods of chemical analysis and in vitro gas production.

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## 1. Introduction

Both milk yield and growth of ruminants are largely limited by forage quality which is mainly reflected in low voluntary intake and digestibility (Minson, 1990). Corn (*Zea may* L.) is a very important cereal crop in the world and Egypt. It is used as food, feed and industrial crop. Nowadays, it becomes a major source for silage for feeding cattle during the summer season in Egypt, because maize produced high green yield and dry matter per unit area, high energy content and quality of its biomass for animal production (Mandic et al., 2015; Roth and Undersander, 1995). Despite variation in nutritive value, maize silage is rich in metabolizable energy and supports higher DMI and milk yield. Harvesting maize silages at DM content between 30 and 35% and feeding in combination with grass silage results in a higher milk yield of dairy cow (Kanak et al., 2013). The importance of using corn plant silage is especially reflected in rations for fattening beef cattle, in which using this nutrient in the amount of 3 to 12 kg/day, with the appropriate mixture of concentrates, significantly contributes to the economy of beef production (Jovanovic et al., 2003). The corn silage-based system is recognized to be standardized owing to a significant bibliography. Dietary corn silage was identified as standard system because it is recognized as strategically to improve sustainability of bovine dairy farm systems. Nutritional quality of the fodder base influences the environmental impact in the buffalo. Also, in dairy buffalo Italian system, good feed efficiency improves sustainability (Bragaglio et al., 2022). Over the last 30 years, corn silage became a popular and nutritious feed for livestock, especially for ruminants (García-Chávez et al., 2022). It is, therefore, increasingly important to choose the correct variety so that silage production can be maximized, resulting in an end product with high yield, high energy, excellent digestibility, and ensuring high animal performance (Allen et al., 1997; Araújo et al., 2012; Bíro et al., 2007; García-Chávez et al., 2022). The Egyptian maize industry's practice of ensiling commercial hybrids and cultivars has resulted in varying variances in the yield generation of digestible nutrients. To maximize profit, however, corn hybrids and types designed explicitly for silage production with a high biomass yield may be chosen during plant breeding programs (Bendary et al., 2001). Hybrid type, cultivation season, stage of maturity and plant density are effective on yield, fermentation characteristics, chemical composition, nutrients digestion, nutritive values and nutrients yields of whole corn plant silage (Gaafar, 2009).

Optimization of harvest maturity, kernel processing, theoretical length of cut, and cutting height improve or maintain the nutritive value of corn silage and milk production of lactating dairy cows. Technological advancements have been developed and made available to dairy producers and corn growers desiring to enhance fiber and starch digestibility of whole-plant corn silage (Ferraretto et al., 2018). The single crossing corn hybrids, white SC 10 and yellow SC 162, are considered the most widely corn hybrids used in making silage in Egypt, due to their high yields of fresh and dry matter, which about 69.1 and 19.78 ton/hectare, respectively for SC 10 (Sayed and El-Nahrawy, 2021) and 81.0-85.8 and 24.3-25.7 ton/hectare for SC 162 (Darwich, 2018). It is possible to determine the fermentation and degradation of feed following the nutritional quality and availability of nutrients for ruminal bacteria using the in vitro gas production technique (Menke and Steingass, 1998) or the modifications by Theodorou et al. (Theodorou et al., 1994) in simulating the digestive processes generated from microbial production (Getachew et al., 1998)

The in vitro semiautomatic gas production measurement technique (Mauricio et al., 1999) is widely used to predict the nutritional value of foods and diets (Lagrange et al., 2019; ZHONG et al., 2016), mainly due to its ability to evaluate many foods, low cost and high repeatability, in addition to being able to describe ruminal fermentation kinetics and estimate the rate and extent of degradation. However, this measurement technique still depends on the manual insertion of a portable pressure transducer into each fermentation bottle, increasing the need for manual operation.

The aim of the study was to determine biomass yield, quality characteristics, chemical composition of yellow and white corn hybrids silage and prediction of their nutritional values using chemical composition and *in vitro* gas production.

## 2. Material and Methods

*2.1 Corn hybrids:*

Single crossings corn hybrids SC 162 (yellow) and SC 10 (white) were closed in this study due to their high biomass yield, which were 2.6-2.7 m for plant height, 2.4-2.6 cm stalk diameter and 117-120 $cm^2$ leaves area per plant. In randomized complete block design, corn hybrids were cultivated at 20 June and grown at a rate of 71,400 plants per hectare using 35 kg of grains during summer season in the north delta of the Kafr El-Sheikh Government in Egypt. This site is 36 meters above sea level and situated at longitude 31 west and latitude 31 north, is thought to provide ideal climatic conditions for the growth of all types of crops due to its high soil quality.

*2.2 Yield determination and silage making:*

To evaluate the production of whole plant corn fodder per hectare, three randomly selected replications plots, each measuring 10 square meters for each hybrid was used to determine the yield of whole plant crop and its parts per hectare. In order to illustrate the relative plant parts, the yield of the ears, stalks, and leaves is also computed as a percentage of the yield of the fresh crop. The fresh silage corn yield was converted to DM based on hectare using the fresh crop's dry matter (DM) content as a percentage (Sade, 1987). After being harvested at the dough stage of maturation (90 days) with DM content 30-35% and chopped using new Holland crop Chopper machine (New Holland Company, Netherland) to maximum length of 1-1.5 cm. Six plastic bags of each hybrid were manually compressed to remove any air and ensiled without additive or inoculants for 35 days in duple bags each containing 80 kg of silage.

*2.3 Silage quality determination:*

The physical quality of finished silage can be rapidly assessed by noting color and odor and samples were taken for chemical analysis. For judging the quality of corn silage, silage extract was prepared by homogenizing 20 g (wet material) of each variety and hybrid silage with 100 ml distilled water in a blender for 10 minutes. The homogenized sample was filtered through a double layer of cheese cloth and the solution was re-filtrated through a filter paper until it become perfectly clears (Waldo and Schultz, 1956). The Orian 680 digital pH meter was used to measure the pH of the silage directly, total volatile fatty acids (TVFAs) were determined using markham micro-distillation apparatus by the steam distillation method Warner (Warner, 1964), and ammonia nitrogen was determined using saturated solution of magnesium oxide distillation according to the method of AOAC (AOAC, 2019). Lactic acid was determined by titration with 0.1 N sodium hydroxide solution using 0.5 ml of phenolphthalein indicator according to the method of James (James, 1995).

*2.4 Chemical analysis of silage sample:*

Using the AOAC method (AOAC, 2019), the sample of silage was dried at 65 °C for 48 hours to measure the dry matter (DM). The dried samples were ground into a powder and used to measure the contents of ash (AOAC method 942.05), crude protein (CP) (AOAC method 2001.11), crude fiber (CF) (AOCS method Ba 6a-05) (AOCS, n.d.), and ether extract

(EE) analysis was carried out as described by AOCS Am 5-04 (Komarek et al., 2004). Both organics matter (OM) and nitrogen free extract (NFE) were calculated by the difference as follows:

OM = 100 – ash (1)

NFE = OM – CP – CF – EE (2)

The Van Soest et al. procedure (Van Soest at al., 1991) was used to determine the NDF, ADF, and ADL contents. Using a normal-phase UV/VIS HPLC the content of β-carotene in silage was determined using Cardinault et al. (Cardinault et al., 2008) methodology and vitamin A using the technique outlined by Tian et al. (Tian et al., 2020)

*2.5 Nutritional values:*

For corn silage, the following total digestible nutrients (TDN) and digestible crude protein (DCP) were computed according to AAFCO (1997):

TDN = 40.2625 + 0.1969 *CP + 0.4228 *NFE + 1.1903 *EE - 0.1379 *CF (3)

DCP = 1.077 * CP – 3/391 (4)

Where: CP = Crude protein; CF = Crude Fiber; EE = Ether Extract; NFE = Nitrogen-Free Extract.

Gross energy (GE) was calculated according to MAFF (1975) and digestible energy (DE) according to AAFCO (1997).

GE (Mcal/kg DM) = (0.226 * CP + 0.177 * CF + 0.407 * EE + 0.192 * NFE)/4.184 (5)

DE (Mcal/kg DM) = (DE * (87.9 – (0.88 * CF))) /100 (6)

*2.6 In vitro study*:

The process outlined by Menke and Steingass (1998) was followed for the *in vitro* gas production evaluation. Briefly, 100 mg of the air-dry feed ingredients were precisely weighed into a 50 ml glass syringe that was calibrated and had plungers *in vitro* gas production, designated as MB9, the buffer solution was employed (Onodera and Henderson, 1980). The buffer comprised 2.8 g of NaCl, 0.1 g of $CaCl_2$, 0.1 MgSO 4.7 $H_2O$, 2.0 g of $KH_2PO_4$, and 6.0 g of $Na_2HPO_4$ that were dissolved in 1 L of distilled water. Next, after adjusting the pH to 6.8, $CO_2$ flushed for 15 minutes. Three rumen-cannulated lambs fed *ad libitum* with a mixture of commercial concentrate and rice straw were used to collect the rumen contents (50 percent liquid and 50 percent solid) (Bueno et al., 2005). Before the animals were fed in the morning, the contents of their rumen were gathered. Both liquids and solids samples were brought to the lab in anaerobic conditions after being placed in insulated flasks preheated to 39 °C. Squeezed through four cheesecloth layers, the rumen contents were maintained in a water bath with $CO_2$ saturation at 39 °C until inoculation. In a water bath held at 39 °C with $CO_2$ saturation, the buffer and inoculant (2:1 v/v) were combined (Nasser et al., 2006; Sallam, 2005; Soliva et al., 2005). After pipetting 15 ml of buffered rumen fluid containing the feed samples into each syringe, the syringes are dropped right into the water bath, which is set at 39 °C. For every experiment, three runs were carried out. Each run consisted of two syringes filled with buffered rumen fluid, which were then incubated and used as the blank. The syringes are gently shaken every two hours, and the incubation is stopped when the 96-hour gas volume is recorded. The gas generation was measured after incubation for 3, 6, 9, 12, 24, 48, 72 and 96 hours. To ascertain the methane ($CH_4$) concentration, 10 µl of the headspace gas was extracted from the bottles at various incubation durations (3, 6, 9, 12, 24, 48,72 and 96 hours) and injected straight into a GC, following the procedure outlined by Pellikaan et al. (2011). The reported gas and methane readings are expressed per 200 mg of DM, and the total gas values are adjusted for the blank incubation. Ørskov and McDonald (1979) defined fermentation kinetics as follows:

$Y = a + b * (1 – e^{-ct})$ (7)

Where: *a* is the gas production from the immediately soluble fraction; *b* is the gas production from the insoluble fraction; *c* is the gas production rate constant for fraction *b*. Y is the gas production (ml/g OM) at time (t).

Gas produced after 3 hours (GP3) of incubation was employed as a new method to measure gas production induced by fermentation of the soluble fraction (GPSF) to evaluate feeds from those parameters. According to van Gelder et al. (2005), the gas produced between the hours of GP3 and GP24 of incubation might be utilized to estimate the gas production due to fermentation of the insoluble fraction (GPNSF) as follows:

$GPSF = (GP_{3hr} * 0.99) – 3$ (8)

$GPNSF = 1.02 + (GP_{24hr} – GP_{3hr}) + 2$ (9)

Where: GPSF is the gas production from a soluble fraction (ml/g DM); GPNSF is the gas production from a non-soluble fraction (ml/g DM); and GP 3hr is the net gas production (ml/200 mg DM) during the three hours.

The amount of gas generated after a 24-hour incubation period was used to compute the energy values, along with additional examinations of crude protein, ash, and fat. Equations given below were used to determine the feedstock used in this approach, created by a research group in Hohenheim, Germany, based on extensive *in vitro* incubation (Menke et al., 1979; Menke and Steingass, 1998).

ME (Mcal/kg DM) = $(2.2 + 0.136 * GP_{24hr} + 0.057 * CP + 0.0029 * CF^2)/4.184$ (10)

NE (Mcal/ kg DM) = $(2.2 + 0.136 * GP_{24hr} + 0.057 * CP + 0.0029 * CF^2 + 0.149 * EE) + 2.2/14.64$ (11)

Where: GP is the net gas production during 24 hours (ml/200 mg DM), CP is a crude protein (% of DM), EE is ether extract (% of DM), and I is the metabolizable energy (Mcal/kg DM).

According to Menke and Steingass (1998), OMD was computed as follows:

$$OMD (\%) = 14.88 + 0.889 * GP_{24hr} + 0.45 * CP + 0.651 * ash \quad (12)$$

Where: GP is net gas production (ml/200 mg DM) after 24 hours, OMD is organic matter digestibility (%), CP is crude protein (% of DM), and Ash (% of DM).

Fig P (Biosoft, Cambridge, UK) is a computer package tool that was used to estimate the degradation kinetics. The Ørskov and McDonald (1979) equation was used to determine the Effective DM Degradability (EDMD):

$$IVDMD = (a + b) + (b*c/c + k) \quad (13)$$

Where: k is the rumen outflow rate, which is at the maintenance level and is equal to 2% per hour, and a, b, and c are the gas production fractions and rates.

The following formula was used to determine short chain fatty acids (SCFA) in accordance with Getachew et al. (2005):

$$SCFA (Mm) = (-0.00425 + 0.0222 * GP_{24hr}) * 100 \quad (14)$$

Where: GP is the soluble fraction's net gas production over 24 hours (ml).

The expected dry matter intake (DMI) of silage was computed using the formula below, in accordance with Blümmel and Ørskove (1993):

$$DMI = 1.66 + 0.49 * a + 0.0297 * b - 4 * c \quad (15)$$

Where: *c* is the gas production rate (ml/hr), *a* is the gas production from the soluble fraction (ml), and *b* is the gas production from the insoluble fraction (ml).

Czerkawski (1986)calculated the generation of microbial protein (MP) as 19.3 g microbial nitrogen per kilogram OMD.

$$MP (g/kg\ DM) = OMD * 19.3 * 6.25/100 \quad (16)$$

*2.7. Statistical analysis:*

The statistical analysis was performed using independent two samples T-test were performed using IBM SPSS Statistics (2014)was used for comparison between the two corn hybrid types. A significant difference was considered as $p < 0.05$.

## 3. Results

*1. Biomass yield of yellow and white hybrids*

Table 1 displays the value related to the main characteristics of the biomass yield. In particular, the yield of corn harvests and the production of relative components and nutrients were considered. For yellow and white corn hybrids, the yields of fresh and dried corn crops were almost the same with differences of 1.67 and 0.79 tons/hectare, respectively. The relative plant parts (ears, stalks, and leaves) also did not exhibit any appreciable variations between hybridized yellow and white corn. When comparing the yellow corn hybrid to the white corn hybrid, the percentage of ears dropped by 1.55%, but the percentages of stalks and leaves increased by 1.00 and 0.55%, respectively. The nutritional yield data indicated that there was a slight difference, although not significant ($p > 0.05$), in the yield of crude protein (CP), digestible crude protein (DCP), and total digestible nutrients (TDN) between the silage of hybridized yellow and white corn. When comparing yellow to white corn hybrid silage, TDN, CP, and DCP yield rose by 0.25, 0.12, and 0.10 tons/hectare, respectively. Also, GE and DE were rose by 3390 and 1600 Mcal/hectare, respectively.

**Table 1.** Biomass **y**ield of yellow and white corn hybrids.

| Items | Yellow hybrid | White hybrid | MSE | *p-value* |
|---|---|---|---|---|
| Crop yield (ton/hectare) | | | | |
| Fresh yield | 71.76 | 70.09 | 0.91 | 0.421 |
| Dry yield | 23.84 | 23.05 | 0.56 | 0.542 |
| Relative parts (%) | | | | |
| Ears | 33.85 | 35.40 | 0.51 | 0.143 |
| Stalks | 45.90 | 44.90 | 0.54 | 0.418 |
| Leaves | 20.25 | 19.70 | 0.25 | 0.324 |
| Nutrient's yield per hectare | | | | |
| TDN (ton) | 15.58 | 15.17 | 0.24 | 0.657 |
| CP (ton) | 2.00 | 1.88 | 0.07 | 0.467 |
| DCP (ton) | 1.34 | 1.24 | 0.05 | 0.365 |
| GE (Mcal) | 105320 | 101930 | 2426 | 0.546 |
| DE (Mcal) | 68053 | 66453 | 1284 | 0.593 |

Abbreviation: The table shows the corn crop yield, relative plant parts, and yellow and white hybrid yield. Abbreviation: TDN: Total digestible nutrient; CP: Crude Protein; DCP: Digestible crude protein, GE: Gross energy; DE: Digestible energy.

*2. Silage quality characteristics*

Organoleptic characteristics were invariably used to judge silage quality as they were practical. Silages made from hybrids of yellow and white corn were observed, and the results showed that the tested silages were characterized with suitable fermentation characteristics, yellowish green color, good smell and free from moldy. The fermentation properties of the corn hybrid silage displayed in Table 2 did not reveal any appreciable variations in the amounts of ammonia nitrogen ($NH_3$-N), lactic acid, and total volatile fatty acids (TVFAs), suggesting a good quality silage.

**Table 2.** Silage quality characteristics of yellow and white corn hybrids silage.

| Items | Yellow hybrid | White hybrid | MSE | *p-value* |
|---|---|---|---|---|
| pH value | 4.15 | 4.10 | 0.05 | 0.854 |
| Lactic acid % of DM | 4.52 | 4.62 | 0.05 | 0.482 |
| TVFA's % of DM | 2.95 | 2.86 | 0.04 | 0.284 |
| $NH_3$-N % of DM | 0.042 | 0.041 | 0.001 | 0.336 |
| $NH_3$-N % of total N | 5.02 | 5.03 | 0.01 | 0.536 |

Abbreviation: DM: Dry Matter; TVFA: Total Volatile Fatty Acid, $NH_3$-N: ammonia nitrogen.

*3. Chemical composition*

Table 3 presents the results of the chemical composition analysis of corn silage. The silage of hybrid yellow and white maize showed negligible variations in the levels of all nutrients with except beta carotene and vitamin A. Comparing yellow corn hybrid silage to white corn hybrid silage, the contents of dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE), and ash tended to increase, whereas organic matter (OM) and Nitrogen-Free Extract (NFE) tended to decrease. In particular, the fiber fraction showed that there were negligible variations between the contents of yellow corn hybrid silage and white corn hybrid silage concerning the amounts of acid detergent fiber (ADF), neutral detergent fiber (NDF), and acid detergent lignin (ADL). Also, the vitamins content of the two hybrids were taken into consideration. In this regard, the yellow corn hybrid silage had considerably more significant levels of β-carotene and vitamin A ($p < 0.001$) than white corn hybrid silage (Table 3). For the nutritional values, when comparing yellow corn hybrid silage to white corn hybrid silage, there was a minor decrease in TDN, GE and DE values and a slight increase in DCP value. These variations were not statistically significant, coming in at 1.11%, 0.004 and 0.029 Mcal and 0.23%, respectively (Table 3). Values of TDN, GE and DE increased with increasing NFE content with strong positive correlations (r = 0.98, 93 and 0.96, respectively) and regression coefficients ($R^2$ = 0.96, 90 and 0.94, respectively), however it decreased with increasing CF content with high negative correlations (r = - 0.92, - 0.85 and - 0.89, respectively) and regression coefficients ($R^2$ = 0.90, 84 and 0.86, respectively). Moreover, DCP value increased with increasing CP content with a high positive correlation (r = 0.98) and regression coefficient ($R^2$ = 0.95).

**Table 3.** Chemical composition, fiber fractions, vitamins and nutritional values of yellow and white corn hybrids silage.

| Items | Yellow hybrid | White hybrid | MSE | *p-value* |
|---|---|---|---|---|
| DM (%) | 33.20 | 32.86 | 0.37 | 0.696 |
| Composition of DM (%) | | | | |
| OM | 93.60 | 93.73 | 0.08 | 0.357 |
| CP | 8.37 | 8.15 | 0.10 | 0.338 |
| CF | 26.43 | 25.77 | 0.32 | 0.365 |
| EE | 2.92 | 2.85 | 0.03 | 0.365 |
| NFE | 55.88 | 56.99 | 0.54 | 0.358 |
| Ash | 6.40 | 6.24 | 0.08 | 0.357 |
| Fiber fractions (%) | | | | |
| NDF | 49.60 | 47.85 | 0.66 | 0.217 |
| ADF | 29.70 | 28.35 | 0.44 | 0.130 |
| ADL | 5.70 | 5.48 | 0.08 | 0.183 |
| Vitamins | | | | |
| β-carotene (mg/kg DM) | 18.47[a] | 15.20[b] | 0.75 | 0.001 |
| Vit. A (IU/kg DM) | 7760[a] | 5850[b] | 441 | 0.001 |
| Nutritional values | | | | |
| TDN (%) | 65.37 | 65.80 | 0.52 | 0.342 |
| DCP (%) | 5.62 | 5.39 | 0.08 | 0.154 |
| GE (Mcal/kg DM) | 4.419 | 4.423 | 0.002 | 0.364 |
| DE (Mcal/kg DM) | 2.856 | 2.885 | 0.014 | 0.365 |

a,b Values in the same row with different lowercase letters differ significantly at p < 0.001.

Abbreviation: DM: Dry matter; OM: Organic Matter; CP: Crude Protein; CF: Crude Fiber; EE: Ether Extract; NFE: Nitrogen-Free Extract; NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber; ADL: Acid detergent lignin; TDN: Total Digestible Nutrient; DCP: Digestible Crude Protein; GE: Gross energy; DE: Digestible energy.

*4. Gas production*

4.1. Cumulative gas production

Values in Table 4 and Figure 1 displayed the cumulative gas outputs for the hybridized yellow and white maize silage. Gas production for both hybrids increased sharply during the first 12 hours of incubation then markedly during the period from 12 to 24 hours and grew progressively as the incubation period extended to 96 hours. At the various incubation times, yellow and white corn silages produced almost the same amount of gas with no appreciable variations ($p > 0.05$). White corn silage tended to produce more gas than yellow corn silage, possibly due to a slight increase in NFE content and a slight decrease in CF and fiber fractions (Table 3).

**Table 4.** *In vitro* gas production (ml/ 200 mg DM) of yellow and white corn hybrids silage.

| Incubation time (hours) | Yellow hybrid | White hybrid | MSE | *p-value* |
|---|---|---|---|---|
| 3 | 15.22 | 15.83 | 0.22 | 0.186 |
| 6 | 23.43 | 24.37 | 0.34 | 0.184 |
| 9 | 29.72 | 30.91 | 0.42 | 0.187 |
| 12 | 37.69 | 39.20 | 0.54 | 0.185 |
| 24 | 50.40 | 52.59 | 0.75 | 0.157 |
| 48 | 58.23 | 60.56 | 0.83 | 0.184 |
| 72 | 62.12 | 64.60 | 0.89 | 0.185 |
| 96 | 64.76 | 67.35 | 0.93 | 0.185 |

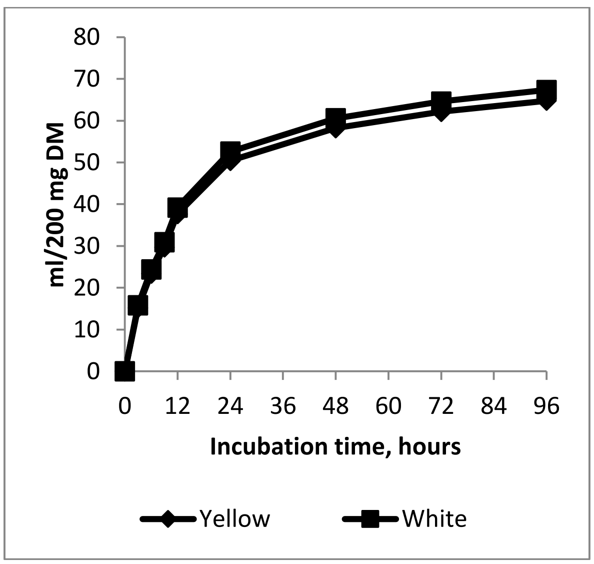


**Figure 1.** *In vitro* gas production of yellow and white hybrids corn silage.

4.2. Gas production fractions

The gas production disclosed only minor variations between the immediately soluble fraction (*a*) and insoluble fraction (*b*), as well as the gas production rate (c) that remains constant for the insoluble fraction (Table 5). Also, for the gas produced during the fermentation of the soluble fraction (GPSF) and insoluble fraction (GPNSF) of the yellow and white corn silages, there was no significant difference ($p > 0.05$). Compared to the gas produced from the soluble fraction, maize silage's insoluble component had more gas (Table 5). High positive correlation between GPSF and gas production at 3 hours of incubation (r = 0.98) and regression coefficient ($R^2$ = 0.94). The corresponding correlation between GPSF and gas production at 24 hours of incubation (r = 0.97) and regression coefficient ($R^2$ = 0.95). Concentration of short chain fatty acids (SCFA) of *in vitro* fermented yellow and white corn hybrids silage was nearly similar with insignificant difference (Table 5). Strong positive correlation between SCFA and gas production at 24 hours of incubation (r = 0.98) and regression coefficient ($R^2$ = 0.96).

**Table 5.** Fractions and rate of gas production of yellow and white corn hybrids silage.

| | Yellow hybrid | White hybrid | MSE | p-value |
|---|---|---|---|---|
| Gas production | | | | |
| *a* (ml/g DM) | 6.39 | 6.65 | 0.09 | 0.188 |
| *b*(ml/g DM) | 57.97 | 60.29 | 0.83 | 0.186 |
| *c* (ml/hour) | 0.058 | 0.061 | 0.001 | 0.104 |
| Gas production during fermentation (ml/g DM) | | | | |
| GPSF | 12.09 | 12.67 | 0.22 | 0.186 |
| GPNSF | 37.88 | 39.50 | 0.54 | 0.146 |
| Short-chain fatty acid (mM/L) | | | | |
| SCFA | 111.46 | 116.33 | 1.66 | 0.157 |

Abbreviation: DM: Dry Matter; *a*: soluble fraction; *b*: insoluble fraction; *c*: gas production rate GPSF: gas produced during the fermentation of the soluble fraction GPNSF: gas produced during the fermentation of the insoluble fraction SCFA: Short-chain fatty acid.

4.3. Methane production ($CH_4$)

Values in Table 6 and Figure 2 showed the methane ($CH_4$) production of corn hybrids silage. Methane production for both hybrids increased sharply during the first 12 hours of incubation then markedly during the period from 12 to 24 hours and grew progressively as the incubation period extended to 96 hours. Across the various incubation periods, the $CH_4$ concentration in the silage of hybrid yellow and white maize was almost the same, with no discernible variations. Yellow corn silage tended to produce less $CH_4$ than white corn silage, possibly due to a slight increase in CF and fiber fractions contents and a slight decrease in NFE content (Table 3).

**Table 6.** *In vitro* methane production (ml/ 200 mg DM) of yellow and white corn hybrids silage.

| Incubation time (hours) | Yellow hybrid | White hybrid | MSE | *p-value* |
|---|---|---|---|---|
| 3 | 1.25 | 1.30 | 0.02 | 0.198 |
| 6 | 1.91 | 1.99 | 0.07 | 0.193 |
| 9 | 2.41 | 2.50 | 0.03 | 0.196 |
| 12 | 3.03 | 3.16 | 0.04 | 0.190 |
| 24 | 4.03 | 4.21 | 0.06 | 0.164 |
| 48 | 4.63 | 4.81 | 0.07 | 0.192 |
| 72 | 4.91 | 5.10 | 0.07 | 0.189 |
| 96 | 5.08 | 5.29 | 0.07 | 0.191 |

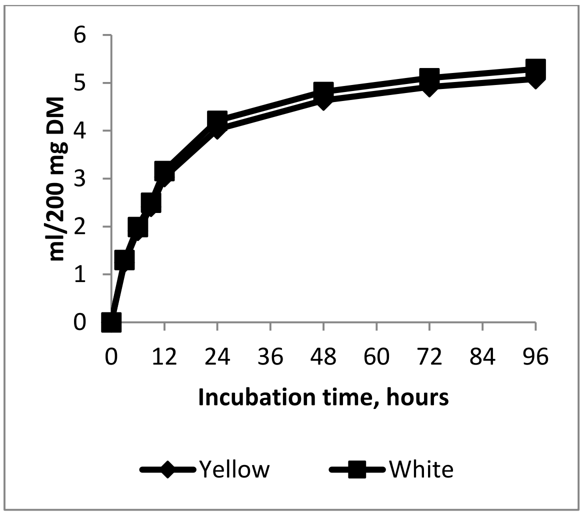


**Figure 2.** *In vitro* methane production of yellow and white hybrids corn silage.

*5. Prediction of Dry matter intake (DMI), organic matter digestibility (OMD), and dry matter degradability (IVDMD)*

Based on the results presented in Table 7, corn silage's in vitro gas generation could be a valuable indicator of its potential for voluntary silage intake when fed on its own or in combination with other meals. With no discernible difference, the estimated DMI for silage made from hybridized yellow and white maize was 6.18 and 6.36 kg/day. Also, for OMD and IVDMD, no significant changes between the hybridized yellow and white corn silages were observed (Table 7). High positive correlation between OMD and gas production at 24 hours was r = 0.98 and regression coefficient was $R^2$ = 0.96. Strong positive correlations among soluble fraction (*a*) and insoluble fraction (*b*) and DMI and IVDMD were r = 0.97, 0.94; 0.95 and 0.93, respectively and the regression coefficients were $R^2$ = 0.93, 0.90; 0.91 and 0.88, respectively.

*6. Metabolizable energy (ME) and net energy (NE)*

The metabolizable energy (ME) from gas production for corn hybrid silage was calculated. The obtained results, 2.76 and 2.81 Mcal/Kg DM, respectively, for yellow and white maize disclosed no significant difference ($p > 0.05$). In the same way, net energy (NE) revealed values of 1.50 and 1.54 Mcal/Kg DM for yellow and white maize ($p > 0.05$). Strong positive correlations between both ME and NE and gas production at 24 hours were r = 0.098 and 0.97, respectively and regression coefficients were $R^2$ = 0.92 and 0.90, respectively.

**Table 7.** Dry matter intake (DMI), organic matter digestibility (OMD), and in vitro dry matter degradability (IVDMD) of yellow and white corn hybrids silage

| Items | Yellow hybrid | White hybrid | MSE | p-value |
|---|---|---|---|---|
| DMI (kg/day) | 6.18 | 6.36 | 0.06 | 0.194 |
| OMD (%) | 64.38 | 66.23 | 0.69 | 0.207 |
| IVDMD (%) | 64.42 | 66.99 | 0.92 | 0.186 |

Abbreviation: DMI: Dry matter; OMD: Organic matter digestibility; IVDMD: in vitro dry matter degradability

*7. Microbial protein*

The microbial protein yield (MP) for the silage of hybridized yellow and white maize was almost the same, at 77.65 and 79.89 g/kg DMI, respectively ($p > 0.05$). Enhancing the production of rumen microbial protein (MCP) is crucial for improving animal performance and lowering waste from a protein diet. High positive correlation between MP and gas production at 24 hours was r = 0.97 and regression coefficient was $R^2$ = 0.94.

## 4. Discussions

*4.1 Crop yield of yellow and white hybrids*

Results obtained from the yellow and white hybrid crop analysis disclose some minor differences. As reported in other studies, this could be due to variations in heritable variance and genetic factors affecting forage yield and its qualities among hybrid maize varieties (Mandic et al., 2015; Seadh S.E. et al., 2014; Tóthné, 2011). Significant genotype variations were observed for the total fresh and dry maize forage yields by Mousa et al. (2017). Bendary et al. (2001) observed that the production of fresh corn silage varied between 33.32 and 56.41 tons per hectare, while the yield of dry matter corn silage varied between 8.83 and 17.30 tons per hectare. Comparable findings were reported by Gaafar et al. (2020), who discovered that the fresh yield of corn silage varied between 60.71 and 72.95 tons/hectare, while the dry yield ran between 19.42 and 25.13 tons/hectare. Also, our findings related to the relative components agreed with the literature. Bendary et al. (2001)reported that the different hybridizations could be responsible for the variations in the comparable features. At the same time, whole plant dry matter (DM) yields and the ratio of grains to stalks are significant factors in determining a corn hybrid's suitability for silage production (Mcdonald et al., 1991). The results of this study are consistent with those of Gaafar et al. (2020), who found that the ranges of values for the ear content, stalk content, and leaf content of corn crops were 29.84 to 36.60%, 46.71 to 52.47%, and 16.64 to 18.65%, respectively. Dry matter yield is a crucial characteristic used in the selection of hybrids of maize used for silage, according to Melchinger et al. (2016). According to Gaafar et al. (2020), the corn silage's TDN yield ranged from 12.97 to 16.30 tons per hectare, its CP yield from 1.55 to 2.05 tons per hectare, and its DCP production from 0.98 to 1.36 tons per hectare.

*4.2. Silage fermentation characteristics:*

The observation of the silages made from yellow and white corn hybrids results in line with those reported in the literature. Church (1991) stated that effective silage treatments had a firm texture, a yellow-greenish tint, and an odor of lactic acid. Therefore, these silage fermentation characteristics were an index of good quality silage for yellow and white maize hybrids. According to Kung and Shaver (2001), typical corn silage will have a pH of 3.7 to 4.2, 4% to 7% lactic acid, 1% to 3% total volatile fatty acids, and 5% to 7% ammonia-N. Our findings both fell between 3.80 and 4.20 on the pH scale, which is often indicative of higher lactic acid concentration and better silage fermentation throughout the ensiling process (Shao et al., 2005). The most common organic acid produced by perfect fermentation, lactic acid is created when sugar is fermented by intestinal and heterofermentative lactic bacteria (Broberg et al., 2007); our results fell below the suggested range of 4-6% (NRC, 2015). Furthermore, the $NH_3$-N/TN shows that the CP degradation is much less than 10%, indicating that the two corn silages in the current study had good fermentation (Bragaglio et al., 2022).

*4.3. Chemical composition*

Considering the relative proportion of the plant, it is a perfect match between the relative proportion of the stalks and the content of fiber fractions. These findings are consistent with those of Gaafar et al. (2020), who discovered a positive association between the proportion of stalks and the fiber fraction contents. According to Roth and Heinrichs (2001), the ranges for the ADF and NDF contents in maize silage are 23.6-33.2 and 41.0-54.1%, respectively. Low levels of corn silage NDF, ADF, and ADL are preferred. Corn silage NDF concentrations vary from 36 to 50%, ADF concentrations from 18 to 26%, and lignin content is low, ranging from roughly 2 to 4% (Rankin, 2023). According to Gaafar et al. (2020), silage from various corn genotypes varied somewhat regarding fiber fractions. Also, other nutrient's composition closely aligns with the respective plant components. Gaafar (2001) and Bendary et al. (2001) observed similar changes in the chemical composition of several corn hybrids, attributed to variances in relative plant components. According to Roth and Heinrichs (2001), the crude protein (CP) concentrations in maize silage ranged from 7.2% to 10.0%. The levels of ether extract (EE), ash, and crude fiber (CF) in corn silage were reported by McDonald et al. (2002)to be 23.3, 5.7, and 10.0%, respectively. Furthermore, the genotype of the plant influences the variations in crude fiber (CF) and crude protein (CP) (Roth et al., 1970). If the main nutritional components remain similar between the yellow and white hybrid corn, the evaluation of vitamins disclosed an increase in β-carotene and vitamin A levels in the yellow corn hybrid silage. This can be due to the 4 mg/kg of β-carotene (Muzhingi et al., 2011) and 2140 IU/kg of vitamin A (USAD, 2019) found in yellow grains. This finding is important from a nutritional point of view. To survive, all vertebrate species require vitamin A. Dietary preformed vitamin A or ppr-vitamin A carotenoids, of which β-carotene is the most significant, can satisfy the need for vitamin A (Green and Fascetti, 2016) since, according to Pickworth et al. (2012), corn silage had a mean β-carotene concentration of 1722 µg/100 g DM and a mean vitamin A content of 6900 IU/kg DM.

Concerning the nutritional values, the results of this study are consistent with those of Bendary et al. (2001), who reported that the TDN value ranged from 62.80 to 72.54% and the DCP value ranged from 4.51 to 6.22% for several corn hybrid silages. Therefore, according to Gaafar (2009), it is preferable to cultivate white corn hybrids for human nutrition rather than yellow corn hybrids for silage. It should be planted in the summer with a plant density of roughly 71,400 plants per hectare and harvested at the dough stage of maturity to achieve the higher yield of silage crop, nutritional values, and digestible nutrients.

*4.4. Gas production*

By incubating substrate in buffered rumen fluid, in vitro gas production (GP) assessment is primarily used to assess the nutritional value of bovine feeds (Cone et al., 1996; Dijkstra et al., 2005;

Getachew et al., 2003). When feedstock is incubated in vitro with buffered rumen fluid, the carbohydrates are fermented to produce short-chain fatty acids (SCFA), microbial cells, and mainly CO and CH gases. The fermentation of carbohydrates to acetate, propionate, and butyrate produces gas. Compared to the fermentation of carbohydrates, the creation of gas from protein fermentation is comparatively minor, and the contribution of fat to gas production is insignificant (Beuvink and Spoelstra, 1992; Ørskov and McDonald, 1979). The complete activity of cellulolytic bacteria, which causes high rates of microbial colonization of the substrate and leads to an efficient energy utilization of the evaluated feeds, depends critically on the quality of the fiber (Sun et al., 2007). According to Silva and Ørskov (1988), a rapidly digestible cellulose and hemicellulose source enhanced the fibrolytic microbes in the rumen. It may, therefore, improve the digestion of other sources of less degradable fiber. According to Liu et al. (2002), gas generation is not necessarily positively correlated with microbial mass production and indirectly indicates substrate degradation. We can determine the fermentation and degradation of food following the nutritional quality and availability of nutrients for ruminal bacteria using the in vitro gas production technique (Menke and Steingass, 1998) or the modifications by Theodorou et al. (1994) in simulating the digestive processes generated from microbial production (Menke and Steingass, 1998). According to Haddi et al. (2003), the rate and degree of GP were significantly correlated negatively with both ADF and NDF.

According to García-Rodriguez et al. (2005) variations in parameters of insoluble fraction (NSF) and gas production rate amongst silages signify distinct fermentation patterns. The features of whole plant corn mentioned above are consistent with the findings that the half-time of the maximum GP of the soluble fraction (SF) increased with increasing maturity, while the asymptotic GP of the soluble fraction and the corresponding maximum gas production rate decreased. Additionally, when the insoluble fraction matured, its maximum gas production rate rose, indicating that starch was more digestible than NDF (Macome et al., 2017). The soluble fraction, insoluble fraction, and degradation rate are the three characteristics that now characterize ruminant forages (Ørskov et al., 1988; Øsrskov, 1991). The soluble fraction represents the water-soluble parts of the dry matter or organic matter, often known as washing loss. According to Ly et al. (1997), it contains the soluble sugars and chemicals released during fermentation, such as polyphenolics. Furthermore, the nutritive value of feeds is evaluated using these characteristics (Ly and Preston, 1997; Øsrskov, 1991).

As for gas production, the concentration of short-chain fatty acids (SCFA) was almost identical, with negligible differences between in vitro fermented silage of hybrid yellow and white corn (Table 5). When feedstock is cultured in vitro with buffered rumen fluid, the carbohydrates undergo fermentation to produce short-chain fatty acids (SCFA) and mainly carbon monoxide and hydrogen gas (Beuvink and Spoelstra, 1992). While feed conversion into short-chain fatty acids (SCFA) and gases are reflected in the gas volume measurement, feed conversion into microbial biomass, short-chain fatty acids (SCFA), and other products of microbial degradation and synthesis is taken into account in the degradability measurement (Grings et al., 2005). Plant components are converted to gas and SCFA by the intricate interactions among a mixed rumen bacteria population (Van Soest, 1994). Getachew et al. (2005), observed a strong correlation between SCFA and the in vitro GP. Based on this relationship, they were able to estimate the generation of SCFA from gas measurements, which measure the animals' energy availability. The generation of SCFA, which was predicated on the fermentation of carbohydrates, was closely correlated with the gas production of various feed classes cultured in vitro in buffered rumen fluid (Kanak et al., 2013; Sallam et al., 2007). McDonald et al. (2002) have revealed that the concentration of SCFA measured in this investigation falls within the usual range of 70 to 150 mM/L.

Soluble fraction, insoluble fraction, and rate of degradation affect the amount of carbon dioxide that ruminants emit into the atmosphere: the ruminal microbiota, the type of carbohydrates supplied, and the amount of feed intake (Johnson and Johnson, 1995; Lascano and Cárdenas, 2010). Due to its effect on propionate, corn hybrids with higher starch contents had reduced CH4 emissions (Aboagye et al., 2019). The type and level of dietary lipids (Grainger and Beauchemin, 2011) and carbs (Ellis et al., 2007) affect the amount of CH4 generated. The ability of various feeding techniques to reduce CH4 production can also be assessed using this in vitro method (Hatew et al., 2015; Holtshausen et al., 2012; Menke et al., 1979).

*4.5. Prediction of Dry matter intake (DMI), organic matter digestibility (OMD), and dry matter degradability (IVDMD)*

The increased intake of corn silage that was projected compared to the other forages indicates how tasty corn silage is. Low digestibility, caused mainly by the concentration of cell wall elements, limits the amount of forage consumed (Blümmel and Becker, 1997; Mould, 2003). High correlations between DMI of forages and in vitro GP investigations have been reported by several authors (Hetta et al., 2007).

The prediction of in vivo OMD was found to be highly precise by Menke et al. (1979), McLeod and Minson (1971), and Van Soest (1994) using the in vitro gas measurement and chemical composition in multiple regression equations. When whole plant maize reached an advanced stage of maturity for harvest, both the effective rumen degradability and the total-tract digestibility of OM declined (Hatew et al., 2016). It is commonly recognized that the most suitable and widely used laboratory methodology for estimating the digestibility of feedstuffs for ruminants is the in vitro technique (Fahey and Hussein, 1999; Tilley and Terry, 1963). It follows that the majority of the data on corn silage quality that is now accessible was gathered using this methodology. It is essential to consider that the samples used in this process are incubated in the test tube for 48 hours, which is longer than the 24-hour retention period during which feed silage particles are subjected to microbial attack in vivo. According to Taghizadeh et al. (Taghizadeh et al., 2006), there is a significant positive link between gas production and maize silage's in vitro dry matter disappearances.

*4.6. Metabolizable energy (ME) and net energy (NE)*

The metabolizable energy value of traditional feeds assessed in vivo showed a positive association with the protein and fiber contents and the metabolizable energy computed from a 24-hour in vitro gas generation(Theodorou et al., 1994). The energy value of various feed classes, especially straws [103], has also been extensively assessed using the in vitro gas production method (Mauricio et al., 1999).

*4.7. Microbial protein*

The quantity of protein nitrogen absorbed into rumen MCP is significantly influenced by the energy provided to rumen microbes. According to Lu et al. (Lu et al., 2019), rumen MCP output increases significantly when fed an energy-rich diet while remaining unaffected by a protein-rich diet. The main variables influencing the energy available for rumen microbial development (namely, MCP synthesis) are the sources and amounts of fed carbohydrates, while provided protein has an impact on the amount of microbial dry matter (DM) produced per unit of fermented carbohydrates (Hoover and Stokes, 1991). To optimize the ruminal synthesis of MCP in dairy cows, a protein level of 12–13% is required (Satter and Roffler, 1975). Only when more non-fiber carbohydrates (NFC), known as the primary energy source for ruminal microbes, are supplied to the animals will more protein N be absorbed into the rumen MCP (Schwab et al., 2005). Between 60 and 85 percent of the amino acids (AA) that enter the animal's small intestine comes from this microbial protein (Storm et al., 1983). Over 80% of the digested rumen MCP makes up 50–80% of the digestible protein in the small intestine (Tas et al., 1981).

**Conclusion**

The present study on two different hybrids of corn, yellow SC 162 and white SC 10, revealed that almost all parameters (silage crop and nutrients yield, chemical composition, nutritional values, silage quality and *in vitro* gas production parameters) except vitamin A and β-carotene levels were higher yellow corn hybrid silage than white corn silage. Also, it can predict nutritional values of TDN, DCP, GE and DE, from chemical composition. Moreover, it can predict gas production from soluble and insoluble fractions, short chain of fatty acids, dry matter intake, organic matter digestibility, *in vitro* dry matter degradability, ME and NE contents, and microbial protein production from *in vitro* gas production.

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