JSAES
Journal of Sustainable Agricultural and Environmental Sciences
Print ISSN : 2735-4377
Online ISSN : 2785-9878
Homepage: https://jsaes.journals.ekb.eg/

Research Article

Impact of Partial Replacement of Yellow Corn with Mango Seed Kernel on *In Vitro* Gas Production and Energy Content

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Article info: -
- **Received:** 4 June 2024
- **Revised:** 8 July 2024
- **Accepted:** 3 August 2024
- **Published:** 10 August 2024

Keywords:
Mango seed kernel; Yellow corn; *In vitro;* Gas production; Rumen fermentation.

Abstract:

In order to assess the impact of partially substituting yellow maize with mango seed kernel (MSK) at concentrate feed mixtures at the levels of 0, 25, 50, and 75% for T1, T2, T3 and T4, respectively on in vitro gas production, and energy content. In comparison to yellow corn grain, MS has lower CP and NFE levels and greater CF and EE contents. Additionally, when the MS level increased, the content of CP tended to decrease and the content of ash tended to increase. Conversely, as the MS level increase, the contents of CF and EE rose while the content of NFE decreases. *In vitro* methane (CH_4) and gas production, gas production for soluble and insoluble fractions, and gas productiion rate (c) data showed that T1 recorded significantly (P<0.05) higher gas production than T4 at various incubation times, with non-noticeable differences from T2 and T3. When comparing T1 to T4, the DMI, In vitro dry matter degradability (IVDMD), organic matter digestibility (OMD), metabolizable energy (ME), net energy (NE), and microbial protein (MP) were significantly (P<0.05) greater, while T2 and T3 showed negligible differences. Also, comparing T1 to T4, gas production for gas production from soluble fraction (GPSF), gas production from insoluble fraction (GPNSF), and short chain fatty acids (SCFA) is significantly higher (P<0.05), but it does not differ significantly from T2 or T3. According to the results of the current investigation, the kinetics and in vitro gas and methane generations were not adversely affected by partially substituting soaked mango seed kernel SMSK at levels of 25 and 50% for yellow corn in the concentrate feed mixtures.

1. Introduction

The main reason the mango (*Mangifera indica*) is grown as a major tropical fruit crop is for its pulp. In most producing countries, mango seed which makes up between 25 and 75 % of the fruit has little utilize in food or industry and is thus thrown away. Mango seed kernel (MSK) is a significant source of carbohydrates (58–80%) and includes low levels of fat (6–16%) and proteins (6–13%). Diarra (2014) states that MSK's protein has a respectable required amino acid profile and that its oil is a rich source of linoleic and stearic acids. The nutritional value of the roughages and any variations in their potential digestibility and energy contents can be ascertained by the application of the in vitro gas production method (IVGPT) (Sallam, 2005). When food is microbialy fermented, methane, carbon dioxide, and short-chain volatile fatty acids (VFA) are generated. The source of gas observed by IVGPT is either direct fermentation or indirect fermentation brought on by these VFA interacting with the bicarbonate found in the incubation medium. Studies have shown that the gas production profiles produced by IVGPT have a strong relationship with the volatile fertilizer (VFA) produced in the rumen (Blümmel and Ørskov, 1993; Brown et al., 2002 and Rymer and Givens, 2002), the neutral detergent fiber (Herrero and Jessop, 1996), and the disappearance of dry matter (DM) (Prasad et al., 1994). The energy values of straws (Makkar et al., 1999), agro-industrial by-products (Krishna and Gunther, 1987), compound feeds (Aiple et al., 1996), and different tropical feeds (Krishnamoorthy et al., 1995) have all been thoroughly assessed using the in vitro gas production method (Getachew et al., 1998). Menke and Steingass (1988) found a significant relationship between the chemical makeup of meals and the projected *in vitro* gas production at 24 hours in relation to the metabolizable energy (ME) values recorded in vivo.

When feedstuffs are incubated in vitro with buffered rumen fluid, the carbohydrates are digested to produce short chain fatty acids (SCFA) gases, primarily CO and CH, as well as microbiological cells. Essentially, the fermentation of carbohydrates to acetate, propionate, and butyrate produces gas. The creation of gas from the fermentation of proteins is rather little when compared to the fermentation of carbohydrates, and fat has little effect on the production of gas (Beuvink and Spoelstra, 1992; Blümmel and Ørskov, 1993).

The aim of this study was to investigate the effects of partial substitution of mango seed kernels at levels of 0, 25, 50, and 75% for corn grain in concentrate feed mixtures on *in vitro* gas production.

2. Materials and Methods

The Sakha Animal Production Research Station, belonging to of the Animal Production Research Institute, Agricultural Research Center, Egypt, is where the current study was conducted. Here is how the experimental treatments were carried out:

T1- Control: corn grain (CG).
T2- 25% of the (CG) in the CFM was replaced by soaking mango seed kernel (MSK).
T3- 50% of the (CG) in the CFM was replaced by (MSK).
T4- 75% of the (CG) in the CFM was replaced by (MSK).

The formulation of different experimental concentrates is presented in Table 1.

Table (1): Formulation of different CFM' s .

Item	CFM1	CFM2	CFM3	CFM4
Wheat bran	**30**	**30**	**30**	**30**
Yellow corn grain	**30**	**22.5**	**15**	**7.5**
Undicorticated Cotton seed meal	**24**	**24**	**24**	**24**
Soaking mango seeds	**0**	**7.5**	**15**	**22.5**
Undicorticated sunflower meal	**10**	**10**	**10**	**10**
Molasses	**3.5**	**3.5**	**3.5**	**3.5**
Limestone	**1.5**	**1.5**	**1.5**	**1.5**
Common salt	**1**	**1**	**1**	**1**
Total	**100**	**100**	**100**	**100**

Gas production *in vitro* was done in accordance with the protocol outlined by Menke and Steingass (1988). 100 mg of the air-dry feed ingredients were precisely weighed and placed 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 Handerson, 1980). The buffer was made up of 2.8 g of NaCl, 0.1 g of $CaCl_2$, 0.1 $MgSO_4.7H_2O$, 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 were given a commercial concentrate mixture and rice straw ad libitum, and the rumen contents (50 percent liquid and 50 percent solid) were collected (Bueno et al., 2005). Prior to the animals being fed in the morning, the rumen contents were gathered. Both liquids and solids were brought to the lab in anaerobic conditions after being placed in insulated flasks that had been preheated to 39°C. The contents of the rumen were kept in a water bath with CO_2 saturation at 39°C until inoculation, after being squeezed through four layers of cheesecloth. The buffer and inoculant (2:1 v/v) were mixed in a water bath that was kept at 39°C with CO_2 saturation (Sallam, 2005; Soliva et al., 2005).

After pipetting 15 ml of buffered rumen fluid—which contains the feed samples into each syringe, the syringes are dropped right into the 39 °C water bath. For every treatment, three runs were carried out. Two syringes, one for each run, were simply filled with buffered rumen fluid, which was used as the blank and incubated afterward. The syringes are gently shaken every two hours, and the experiment concludes when the gas volume for the whole 96 hours is measured. The syringes are gently shaken every two hours, and the incubation period concludes when the gas volume for the whole 96 hours is measured. The amount of gas generated was measured following 3, 6, 9, 12, 24, 48, 72, and 96 hours of incubation. In order to ascertain the methane (CH_4) concentration, ten microliters of the headspace gas were 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). After incubation for 3, 6, 9, 12, 24, 48, 72, and 96 hours, the gas generation was measured. The reported gas values are provided per 200 mg of DM and the total gas values are adjusted for the blank incubation. The kinetics of fermentation was explained as follows by McDonald and Ørskov (1979):

$$Y = a + b\,(1 - e^{-ct})$$

When c is the gas production rate constant for fraction b, a represents gas production from the immediately soluble fraction and b represents gas production from the insoluble fraction. Gas production (ml/g OM) at time t is represented by Y.

A novel approach to assessing feeds from those parameters was the use of gas produced after three hours (GP3) of incubation, which resulted from the fermentation of the soluble fraction (GPSF). Based on the findings of (Van Gelder et al., 2005), the gas produced between three hours (GP3) and twenty-four hours (GP24) of incubation may be used to estimate the gas production produced by the fermentation of the insoluble fraction (GPNSF) as follows:

GPSF= Gas 3hr * 0.99 **-** 3

GPNSF= 1.02*(Gas 24hr – Gas 3hr) + 2

Where: Fuel In terms of "GPSF" (gas production from soluble fraction) and "GPNSF" (gas production from insoluble fraction), respectively, the phrases "3hr" and "Gas 24hr" denote the quantity of net gas generated in milliliters per 200 milligrams of DM.

The energy values were calculated using the volume of gas produced during a 24-hour incubation period in addition to further analyses of crude protein, ash, and fat. This method was developed by a research group in Hohenheim, Germany, and is based on extended in vitro incubation of the feedstock (Menke et al., 1979 and Menke and Steingass, 1988).

ME (Mcal/kg DM) = (2.2 + 0.136*GP 24hr + 0.057 *CP + 0.0029*CF2)/4.186

NE (Mcal/kg DM) = (2.2 + 0.136*GP 24hr + 0.057 *CP + 0.0029*CF2 + 0.149*EE) *2.2/ 14.64

where ME is the metabolizable energy (Mcal/kg DM), CP is crude protein (% of DM), EE is ether extract (% of DM), and GP is the net gas production during a 24-hour period (ml/200 mg DM).

OMD (%) = 14.88 + 0.889*GP + 0.45*CP + 0.0651*A

Where OMD is the organic matter digestibility (%), CP is the crude protein (% of DM), A is the ash (% of DM), and GP is the net gas production (ml/200 mg DM) after 24 hours. In agreement with Getachew et al. (2002), the short chain fatty acids (SCFA) were calculated using the following formula:

SCFA = (- 0.00425 + 0.0222*GP 24hr) *100

Where: GP is the soluble fraction's net gas production over a 24-hour period (ml).

Following the formula from Blümmel and Ørskove (1993), the dry matter intake (DMI) was determined as follows:

DMI = 1.66 + 0.49 * (a) + 0.0297 * (b) – 4 * (c)

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

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

IVDMD = (a+b) + (bc/(c + k))

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 rate.

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

Statistical analysis was performed on the data using the General linear Models approach, which was modified for the user's guide by SPSS for Windows (2008). To ascertain the level of significance between the means, the Duncan test was used within the SPSS program (Duncan, 1955).

3. Results and Discussions

Chemical composition of different CFM:

Chemical composition in Table 2 revealed lower CP and NFE contents and higher CF and EE content in MS compared to YCG. Also, the content of CP tendered to decrease and ash tended to increase with increasing MS level. Whereas, CF EE contents increased and NFE content decreased with increasing MS level. The current findings concur. with those reported by Sruamsiri and Silman (2009), Diarra (2014), Omer et al. (2019) and Admasu et al. (2020).

Table 2: Chemical composition of YCG, MS and different CFM

Item	YCG	MS	CFM1	CFM2	CFM3	CFM4
DM	**91.58**	**88.33**	**91.23**	**91.08**	**90.95**	**90.82**
OM	**98.70**	**98.21**	**92.84**	**92.81**	**92.78**	**92.75**
CP	**9.29**	**7.13**	**15.98**	**15.94**	**15.90**	**15.85**
CF	**2.10**	**22.21**	**12.25**	**13.74**	**15.24**	**16.73**
EE	**1.70**	**4.81**	**2.90**	**2.98**	**3.06**	**3.13**
NFE	**85.70**	**64.06**	**61.71**	**60.15**	**58.58**	**57.04**
Ash	**1.30**	**1.79**	**7.16**	**7.19**	**7.22**	**7.25**

DM, Dry matter, OM, organic matter; CP, crude protein; EE, ether extract; CF, crude fiber; NFE, nitrogen free extract

Cumulative gas production:

The effects of different levels of Soaking Mango Seed Kernel (SMSK) supplementation on in vitro cumulative gas production of experimental treatments are presented in Table (3), Fig. (1). In vitro gas production data revealed that T1 recorded significantly (P<0.05) the higher gas production at different incubation time compared to T4. Whereas, gas production of T2 and T3 did not significantly (P>0.05) differ with both T1 and T4. So, from these results it can be replaced YCG by MS up to 50% in concentrate feed mixture. Gas production for the different treatments increased markedly during the first 24 hours of incubation and then increased gradually afterwords. These results are in accordance with those obtained by (Blummel and Becker., 1997) they clarified that, in general, the quantity of gas generated depends on the type of carbohydrate consumed since gas production is a function and a mirror of the digestible carbohydrates in the diet. The high gas production for Mango Seed treatments could be regarded to its high contents of soluble ash and readily fermentable carbohydrates. Fondevila et al. (2002) reported negative effects of concentrate high in carbohydrates due to a modification of the environmental conditions of the rumen towards an unfavorable condition for the fibrolytic microorganisms. Whereas supplementation with tree leaves invariably alleviate N deficiency and other mineral deficiencies, thus increasing the intensity of rumen microbial activity (Bonsi et al., 1994; Merkel et al., 1999). According to Hove et al. (2001), the fermentation of carbohydrates into acetate, propionate, and butyrate is the basic cause of gas generation. Getachew et al. (2002) reported that NFC was positively correlated with potential gas production characteristics that suggest that gas production from carbohydrate fermentation is relatively high as compared with protein fermentation. According to Riad et al. (2022), as incubation durations grew, so did the total volume of gas. Throughout all of the incubation periods, there were noticeable variations in the gas generation between the substrates. Thus, gas productions at all incubation times were higher (P<0.05) higher than those of the other substrates.

Table 3: Effect of Impact of adding Soaking Mango Seed Kernel (SMSK) on the total amount of gas produced over various incubation times.

Item	Incubation time (hour)							
	3	6	9	12	24	48	72	96
T1	14.20[a]	20.54[a]	26.54[a]	34.98[a]	46.14[a]	54.30 [a]	57.08[a]	59.75[a]
T2	13.58[ab]	19.79[ab]	25.66[ab]	33.91[ab]	44.61[ab]	52.60[ab]	55.38[ab]	57.67[ab]
T3	12.95[ab]	18.54[ab]	24.79[ab]	32.63[ab]	43.09[ab]	50.90[ab]	53.68[ab]	55.60[ab]
T4	12.33[b]	17.98[b]	23.91[b]	31.46[b]	41.56[b]	49.20[b]	51.98 [b]	53.52[b]
SE	0.603	0.458	0.520	0.386	0.716	0.833	0.752	0.796

a, b: Means with different superscripts in the same column differ significantly (P<0.05).

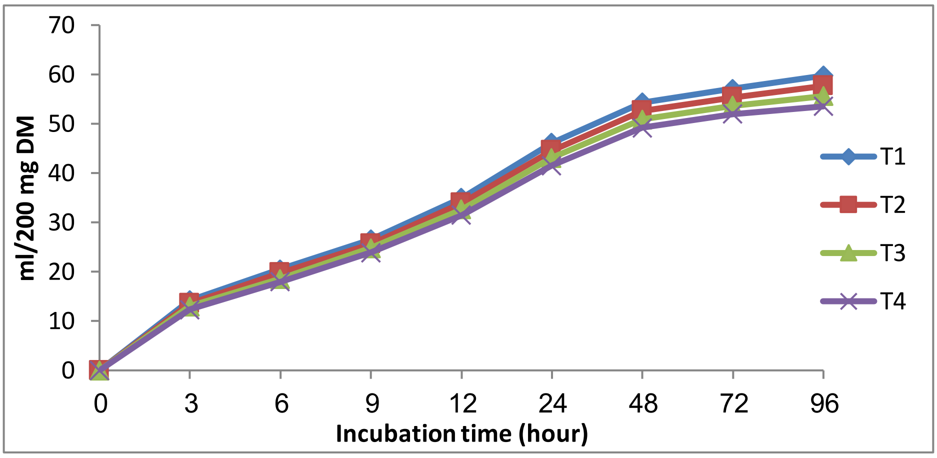

Fig. 1: ***In vitro*** **gas production of different CFM.**

Fractions and rate of gas production:

Table (4) illustrates how the amount of MSK supplementation affects the experimental treatments' fractions and gas generation rate. The soluble fraction (a), insoluble fraction (b), and gas generation rate (c) in T1 were significantly (P<0.05) higher than in T4, with no discernible differences between T2 and T3. The current findings showed that substituting mango seeds for yellow corn resulted in a reduction in overall gas generation. Changes in the chemical makeup were connected to this. The b fraction represents the potentially may escape rumen degradation but absorbed in the rumen (NRC, 1981). The low b value obtained is an indication of the fibrous nature of the feedstuffs incubated. According to Riad et al. (2022), T20's soluble fraction (a) produced considerably (P<0.05) more gas than T40 and T60. For T0 and T40, the amount of gas produced from an insoluble fraction (b) was substantially (P<0.05) higher than that of T20 and T60. But compared to the other substrates, T60's potential gas output (a+b) was noticeably (P<0.05) lower. Gas production and estimated parameters (c, a, b, and a + b) demonstrated a positive correlation with CP, one of the characteristics limiting micro-bial development (Kamalak et al., 2005). When it comes to high protein feedstuffs, the soluble fraction's protein is often fermented quickly at first. This fermentation reaches its peak after 20 hours, and by 46 hours, the protein concentration is likely to befully fermented (Cone and Van Gelder, 1999).

Table 4: Gas production parameters for different supplementing Mango Seed.

Item	T1	T2	T3	T4	SE
a (ml/g DM)	**5.537[a]**	**5.237[ab]**	**4.937[ab]**	**4.637[b]**	**0.535**
b (ml/g DM)	**57.50[a]**	**55.63[ab]**	**53.75[ab]**	**51.88[b]**	**1.08**
c (ml/hour)	**0.06[a]**	**0.05[ab]**	**0.04[ab]**	**0.03[b]**	**0.004**

a, b: Means with different superscripts in the same column differ significantly (P<0.05).

In vitro **methane production**:

The findings shown in Table (5) and Fig. (2) demonstrated that methane production was considerably (P<0.05) greater for T1 in comparison to T4 over the various incubation intervals, whereas there were no significant changes for T2 or T3. Methane production for the different treatments increased markedly during the first 24 hours of incubation and then increased gradually afterwords. The feedstock ferments the carbohydrates to form microbiological cells and short chain fatty acids (SCFA), mainly CO and CH gasses, when it is treated in vitro with buffered rumen fluid. In essence, gas is produced by the fermentation of carbohydrates to acetate, propionate, and butyrate. The creation of gas from the fermentation of proteins is rather little when compared to the fermentation of carbohydrates, and fat has little effect on the production of gas (Beuvink and Spoelstra, 1992; Blummel and Ørskov, 1993). According to Moe and Tyrrel (1979), methane generated per gram of digested cellulose is five times more than methane produced per gram of digested non-fiber carbohydrates, such as starch.

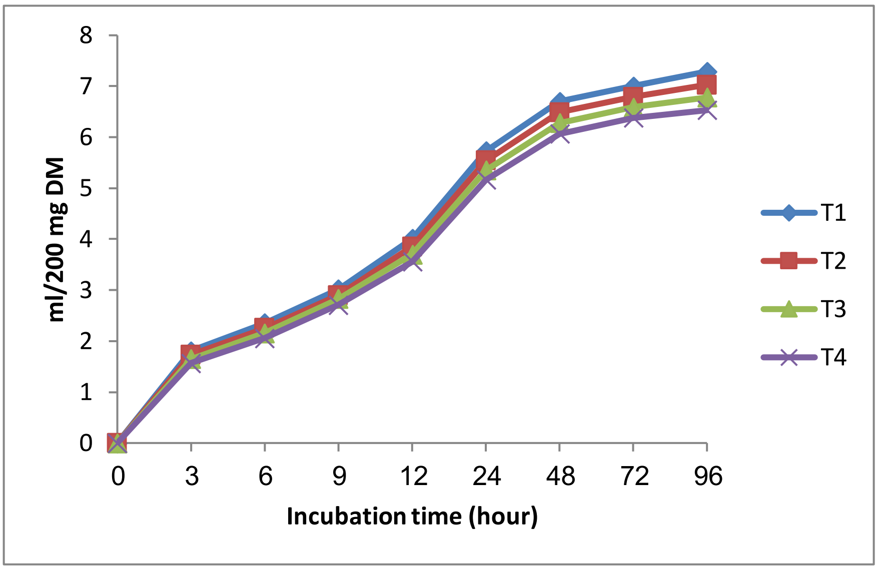

Fig. 2: ***In vitro*** **methane production of yellow and white hybrids corn silage**

Table 5: Effect of supplementing Soaking Mango Seed Kernel (MSK) on the methane production during different incubation time.

Item	**Incubation time (hour)**							
	3	**6**	**9**	**12**	**24**	**48**	**72**	**96**
T1	1.81[a]	2.35[a]	3.02[a]	4.01[a]	5.73[a]	6.70[a]	7.00[a]	7.29[a]
T2	1.73[ab]	2.25[ab]	2.90[ab]	3.85[ab]	5.54[ab]	6.49[ab]	6.79[ab]	7.03[ab]
T3	1.65[ab]	2.16[ab]	2.83[ab]	3.70[ab]	5.35[ab]	6.28[ab]	6.59[ab]	6.78[ab]
T4	1.57[b]	2.06[b]	2.71[b]	3.56[b]	5.16[b]	6.07[b]	6.38[b]	6.53[b]
SE	0.08	0.06	0.07	0.05	0.09	0.10	0.09	0.10

a, b: Means with different superscripts in the same column differ significantly (P<0.05).

Dry matter intake (DMI):

Table 6 results showed that the expected DMI was significantly (P<0.05) higher with T1 compared to T4 (5.84 vs. 5.35 kg/day). While, DMI was nearly similar in all treatments. Low digestibility, which is mostly caused by the concentration of cell wall elements, limits the amount of forage that may be consumed (Blummel and Becker, 1997; Mould, 2003). High correlations between DMI of forages and in vitro GP investigations have been reported by several authors (Blummel and Becker, 1997; Hetta et al., 2007).

In vitro dry matter degradability (IVDMD), Organic matter digestibility (OMD), metabolizable energy (ME) and net energy (NE):

As presented in Table (6), T1 recorded significantly (P<0.05) higher IVDMD, OMD, ME, NE and MP compared to T4 with insignificant differences with both T2 and T3. The differences in effect of In vitro fermentation on organic matter digestibility (OMD) of the browse forages could be as a result of morphological fraction, environmental factor or maturity stage as also observed by (Babayemi et al., 2006).

Because increased fiber intake reduces protein digestibility, which in turn lowers the ME content of the diet, fiber is known to alter the ME content of substrates (Baer et al., 1997).

Menke and Steingass (1988) found a favorable association between the ME value of standard feeds assessed in vivo and the CP and fat content determined from in vitro gas generation. Chenost et al. (1997) came to the conclusion that measurements of gases and chemical constituents yield a more accurate forecast of ME than estimates based just on chemical constituents. Feeds with high levels of lignin and/or acid insoluble ash have poor TDN and OMD scores because indigestible materials like these generally hinder the digestion of other beneficial nutrients. According to Mokoboki et al. (2019), the fiber content of acid detergent and dry matter degradability were negatively correlated. While the data published by Repetto et al. (2003) demonstrated that ADF had the closest link with DMD, this result was consistent with Kamalak et al. (2005). True digestible organic matter (TDOM) and total SCFA concentration showed a negative correlation with NDF and ADF content, and a positive correlation with CP, EE, and non-fiber carbohydrate (NFC) content (Pal et al 2015).

Table 6: Effect of adding mango seed as a supplement on the intake of dry matter, the digestibility of organic matter, the amount of energy that can be metabolized, net energy, and microbial protein.

Item	T1	T2	T3	T4	SE
DMI kg/day	5.84^{a}	5.67^{ab}	5.50^{ab}	5.35^{b}	0.24
INDMD%	64.71^{a}	62.27^{ab}	59.82^{ab}	57.26^{b}	0.71
OMD%	63.56^{a}	62.18^{ab}	60.81^{ab}	59.43^{b}	0.644
ME(Mcal /kg DM)	2.35^{a}	2.32^{ab}	2.30^{ab}	2.29^{b}	0.024
NE(Mcal/kgDM)	1.54^{a}	1.53^{ab}	1.52^{ab}	1.51^{b}	0.014
MP(g/kg DM)	76.66^{a}	75.00^{ab}	73.36^{ab}	71.69^{b}	0.778

a, b: Means with different superscripts in the same column differ significantly (P<0.05).

Gas production from the fermentation of soluble (GPSF), insoluble fractions (GPNSF) and Short chain fatty acids (SCFA):

Table 7 shows the gas generated during the fermentation of the soluble (GPSF) and insoluble (GPNSF) fractions, as well as the concentration of short chain fatty acids (SCFA). When comparing T1 to T4, gas production for GPSF, GPNSF, and SCFA is considerably greater (P<0.05), but it does not change significantly for T2 and T3. Stoichiometric reaction equations have been used to characterize the intricate interactions among a mixed rumen microbial community that result in the conversion of plant components into gas and SCFA (Wolin., 1975, Wolin, 1979, Russell and Wallace., 1988, and Van Soest, 1994). Getachew et al., (2002) observed a strong correlation between SCFA and the in vitro GP. Based on this link, the authors estimated the generation of SCFA from gas values, a measure of the animals' energy availability. Njidda (2010) discovered a strong correlation (R2 = 0.99; P<0.05) between the gas output computed from SCFA and that which was measured in vitro.

Table 7: Effect of supplementing Mango Seed on the formation of gas by the fermentation of short-chain fatty acids, gas CH3, and soluble and insoluble components.

Item	T1	T2	T3	T4	SE
GPSF (ml/g DM)	11.06^{a}	10.44^{ab}	9.82^{ab}	9.21^{b}	1.589
GPNSF(ml/g DM)	34.58^{a}	33.65^{ab}	32.74^{ab}	31.81^{b}	1.019
SCFA (Mm)	102.01^{a}	98.61^{ab}	95.23^{bc}	91.84^{c}	1.589

a, b,c: Means with different superscripts in the same column differ significantly (P<0.05)

4. Conclusions

Based on the current study findings, it can be said that using mango seeds instead of yellow corn grains at levels of 25% or 50% had no negative effects on the parameters of in vitro gas production. Therefore, it can replace up to 50% of the maize grains in the concentrate feed combination with energy source from mango seeds.

Author Contributions: W. R.; A. E. and S.A. plannedand supervised the research. A. S., W.R. and G. E. conducted the experimental work and analyzed the data. W. R.; A. E. and A. S. drafted the manuscript while consulting with every other author.

Funding: "This research received no external funding"

Institutional Review Board Statement: The institutional ethical rules of Agriculture College - Tanta Uversity (No. AY 2019-2020/Session 6/2020.01.13) in the course of the experiment with animals used for scientific reasons.

Informed Consent Statement: "Not applicable"

Data Availability Statement: "Not applicable"

Acknowledgments: The authors are thankful to the lab technicians

Conflicts of Interest: No conflict of interest regarding the publication of this paper.

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