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EFFECT OF PARTIAL REPLACEMENT OF YELLOW CORN BY MANGO SEEDS ON *IN VITRO* RUMEN DEGRADABILITY, ENERGY CONTENT AND METHANE EMISSION

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SUMMARY

*Mango seeds, which are not consumed by human beings, are a potential feed ingredient, a good source of soluble carbohydrate, protein, and higher fat than corn. This present study objectivewas conducted to examine the effect of replacing yellow corn with mango seed soaked in water (MSSW) in the formulated concentrate mixtures and experimental diets on in vitro gas production and ruminal fermentation. Four formulated concentrate mixtures consisted of 0, 20, 40 and 60% MSSW, referred to as T_0, T_{20}, T_{40} and T_{60}, respectively. The experimental diets consist of the previous concentrated mixtures with Egyptian clover hay (**CH**) and/or wheat straw (WS), T_0+CH, T_0+WS, T_{20}+CH, T_{20}+WS, T_{40}+CH, T_{40}+WS, T_{60}+CH and T_{60}+WS, respectively. The effects of replacement were assessed by incubation of the tested substrates in buffered rumen fluid using an in vitro gas production technique**,** Metabolizable energy, net energy lactation, total digestible nutrientsand organic matter digestibility (OMD) were calculated from gas production after 24-h incubation. Microbial protein was calculated from OMD. Methane emissionwas high for T_{60} and T_{60}+WS compared with other tested substrates. In conclusion, the results revealed that replacing up to 60% of corn with MSSW as an alternative energy source could be useful in ruminants feeds.*

Keywords: in vitro, mango seeds, microbial protein, methane emission

INTRODUCTION

The severe competition between man and livestock for feedstuffs has made it difficult to provide enough fodder to meet the needs of producing animals, especially in developing countries, due to insufficient quantities of traditional ingredients used to formulate animal rations. The scarcity of traditional feed sources often imposes a major challenge for livestock production in many countries worldwide. Such a challenge can be alleviated using unconventional feedstuffs in animal feeding depending on their nutritional contents, availability and acceptability to animals. The use of fruit-vegetable wastes like the residues of mango in animal nutrition has attracted many researchers' attention due to their local availability and high nutritionalvalue. The mango residues represent 40-50 % of the fruit weight. They have potential dietarybenefitsin addition to significant levels of essential minerals (Fowomola, 2010).However, large amounts of mango wastes, generated during the industrial processing, create serious environmental pollution problems (El-Kholy *et al*., 2008). Recently, starch from fruit seeds, including mango, was found to possess good physicochemical properties besides, kernel is balanced in amino acids (Hassan *et al*., 2013). Mango seed kernel (MSK) was reported to be a good source of carbohydrates (77%), fat (11%), proteins (6-7%), crude fiber (2%) and ash (2%) on a dry weight basis (Diarra *et al*., 2014). Also, mango kernel contains 170 mg/100 g of calcium, 210 mg/100 g of magnesium, and 368 mg/100 g of potassium (Yatnatti, *et al*, 2014) and rich in provitamin A (15.27 IU), vitamin E (1.30 mg/100 g db), vitamin K (0.59 mg/100 g db), and vitamin C (0.56 mg/100 g db). Other vitamins include vitamin B1, B2, B6, and B12 in quantities of 0.08, 0.03, 0.19, and 0.12 mg/100 g db, respectively (Fowomola, 2010). However, it is fairly rich in tannins and also contains cyanogenic glucosides, oxalates and trypsin inhibitors (Sultana and Ashraf, 2019). Several treatments such as soaking, boiling, HCl or NaOH treatment, autoclaving or HCl followed by $Ca(OH)_2$ were experimented to remove tannins and HCN but soaking proved to be the more effective as it removed 61% of the tannins and 84% of HCN (El Boushy *et al*., 2000). The nutritive value of mango kernels was improved by boiling, and depressed growth due to feeding untreated kernels was restored (Diarra *et al.*, 2010). The metabolizable energy value was low (7.9 MJ/kg DM) in dried raw kernels but have increased up to 10.3 MJ/kg DM after boiling (Ravindran and Sivakanesan, 1996). There are few reports on the use of mango seeds in livestock feeding, so further studies are needed to identify mango seed kerneleffect on rumen fermentation, animal performance, health, and,consequently, on income. The objectives of the present study were to investigate both the effects of partial yellow corn replacement by mango seeds and the type of used roughage on *in vitro* ruminal fermentation, gas production, energy values, volatile fatty acids, and methane production.

MATERIALS AND METHODS

The present study was carried out at the laboratory of rumen microbiology, Animal and Fish Production Dept., Faculty of Agriculture, Alexandria University, Alexandria, Egypt. All procedures and experimental protocols were carried out meeting the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

Feedstuffs:

Mango seeds preparation:

Mango seeds were washed with tap water, soaked in water for 3 days, sundried, thenmango seeds destroyed by cracking the seeds manually, Cracked seeds were ground in mills to pass a 1mm sieve, dried in hot air oven at 60ºC for 4 hours and stored in air tight containers till chemical analys**es**, *in vitro* gas production and rumen fermentation analyses were carried out.

The experimental diets:

The concentrate feed mixtures (Tables 1, 2 and 3) contained 0, 20, 40 and 60% inclusion levels of mango seeds soaked in water (MSSW) as a replacement for corn, T_0, T_{20}, T_{40} and T_{60}, respectively. Also, eight experimental diets were prepairedas follow: 40 g of T_0, T_{20}, T_{40} and T_{60} mixed with 60 g of Egyptian clover hay (*Trifolium alexandrinum*) (CH) and/ or wheat straw (WS), referred to as the T_0+CH and T_0+WS and T_{20}+CH and T_{20}+WS, T_{40}+CH and T_{40}+WS, and T_{60}+CH and T_{60}+WS.

Chemical Analyses:

Representative samples of the experimental diets chemical analyses were conducted according to AOAC (1995) for OM (ID number 942.05), EE (using Soxhlet procedure, ID number 938.06), and CP (as 6.25× N; ID number 954.01). The neutral detergent fibers (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) were determined according to Van Soest*et al*., (1991). Cellulose and hemicelluloses were calculated. The content was calculated as follows: NFE (g/ kg DM) = 100 − (CP + ash + EE + CF).

In vitro gas production and rumen fermentation:

In vitro gas production determination was completed according to the procedure described by Menke and Steingass (1988). Buffer and mineral solutions are prepared and placed in a water bath at 39^{o}C under continuous flushing with CO_2. Rumen fluid collected from three fistulated sheep fed on **Egyptian clover** hay and commercial concentrate mixture diet were collected into a pre-warmed thermos flask. The rumen fluid was filtered through four layers of gauze and flushed with CO_2, and the mixed and CO_2 flushed rumen fluid was added to the buffered mineral solution (1:2 v/v), which was maintained in a water bath at 39^{o}C, and combined. Samples (200±10mg) of the air-dried feedstuffs were accurately weighed into a syringe fitted with plungers. Buffered rumen fluid (30 ml) is pipetted into each syringe, containing the feed samples, and the syringes were immediately placed into the water bath at 39^{o}C (Blümmel and Ørskov, 1993). Three syringes with only buffered rumen fluid were incubated and considered as the blank. The syringes were gently shaken every 2 h, and the incubation was terminated after recording the 96 h gas volume. The gas production was recorded after 3, 6, 9, 12, 24, 48, 72 and 96 h of incubation. Cumulative gas was expressed as milliliter of gas produced per 200 mg of dry matter and corrected for blanks. Cumulative gas production GAS (Y) at time (t) was fitted to the exponential model of Ørskov and McDonald, (1979) as follows:

Gas (Y) = a + b (1-exp^{-ct}),

Where:

a = the gas production from the immediately soluble fraction, **b** = the gas production from the insoluble fraction, **c** = the gas production rate constant for the insoluble fraction (b), **t** = incubation time.

Estimation of energy values, short chain fatty acids, organic matter digestibility and microbial protein:

The energy values and OMD were calculated from the amount of gas produced at 24 h of incubation with supplementary analysis of crude protein; ash and ether extract (Menke *et al*., 1979 and Menke and Steingass, 1988). Short chain fatty acids were predicted according to Getachew *et al*. (2005). Microbial protein was calculated based on 19.3 g microbial nitrogen per kg OMD according to Czerkawaski, (1986). Methane production was measured according to Fievez *et al*. (2005).

For concentrates the following equations were used:

ME (MJ /Kg DM)= 1.06+0.157Gp+0.084CP+ 0.22CF- 0.081CA

OMD (%)= 14.88 + 0.889 Gp + 0.45CP + 0.0651 CA

NEL (MJ/kg DM) = 0.115*GP+ 0.0054*CP+ 0.014*EE- 0.0054 CA-0.36

For roughages the following equations were used:

ME (MJ/kg DM) = 2.20 + 0.1357*Gp + 0.0057 x CP (g/kg DM) + 0.000286*(EE)^2(g/kg DM)

NEL(MJ/kg DM)= 0.0960*Gp +0.0038 x CP (g/kg DM) + 0.000173* (EE)^2(g/kg DM) + 0.54

ME (MCal/kg DM) = ME (MJ/kg DM)/4.184

TDN was calculated from ME value as per the following equation (NRC, 1989):

TDN (%) = [ME (MCal/kg DM) + 0.45] / 0.0445309

Short chain fatty acids (SCFA) were calculated according to Getachew *et al*. (2005) as follows: **SCFA (mM) = (-0.00425+ 0.0222*GP)*100**

Where: GP is 24 h net gas production (ml/200 mg DM), CP is crude protein (%of DM), EE is crude fat (%of DM) and CA is ash (% of DM).

Statistical Analysis:

Data were subjected to analysis of variance (ANOVA) using the General linear Model (GLM). Significant differences between individual means were identified using least significance difference (LSD) multiple range test (SAS, 2000).

RESULTS AND DISCUSSION

Chemical composition of feed ingredients:

Chemical composition of yellow corn, mango seeds soaked in water, Egyptian clover hay, and wheat straw, which were included in both the formulated concentrate mixtures and the total mixed ration, are presented in Table 1. Data illustrated that the values of OM, CP, GE and DE in MSSW are approximately similar to that of yellow corn. Meanwhile, MSSW is superior in their content of EE, CF, NDF, ADF, ADL and CEL in comparison with yellow corn. The ash content in yellow corn (1.30%) is slightly lower than ash content in MSSW (1.75 %), while the NFE, HC and NFC in MSSW were lower than yellow corn. Results for ash, CF and CP content in the current study are different from the results of Admasu *et al.*, 2020, who reported 4.73% ash, 3.98% CF and 8.98% CP contents of boiled mango seed kernel (BMSK). On the other hand, ash values were 1.40 and 2.38 for yellow corn and MSK, respectively (Omer *et al*., 2019). In the present study, the chemical composition of MSSW showed some variations from many authors' reports, which may reflect the different types MSSW or treatments. The present results are in agreement with those noted by Diarra (2014) who reported that MSK is a good source of carbohydrates (67–82%) and contain moderate quantities of proteins (6–10%) and fat (7–14%), while differing from the results of Sruamsiri and Silman (2009), who showed that the chemical composition of mango seeds was 4.19%, 30.84%, 2.72%, 47.79%, 53.01%, 31.20% and 4070 kcal/kg DM for CP, CF, EE, NFE, NDF, ADF and GE, respectively. The current study showed that the values of OM, GE, DE, ADL and ash in BH are approximately similar to that of WS, while CF, NDF, ADF, HC and CEL in WS were higher than CH. The values of CP, EE and NFC were 4.2 and 11.6, 0.83 and 1,9 and 28.51 and 32.37 % for WS and CH, respectively.

Table1. Chemical composition of yellow corn, mango seeds soaked in water, Egyptian clover hay (CH) and wheat straw (WS) (on a dry matter basis)

Items %	Yellow Corn	MSSW	CH	WS
OM	98.70	98.21	90.20	91.10
CP	9.24	7.13	11.60	4.20
EE	1.70	4.81	1.90	0.83
CF	2.10	22.21	31.13	37.16
NFE	85.66	64.06	45.57	48.91
Ash	1.30	1.79	9.80	8.90
NDF	24.82	31.78	44.33	57.56
ADF	4.42	23.49	34.17	38.95
ADL	0.56	7.76	7.06	6.20
HC	20.40	8.29	10.16	18.61
CEL	3.86	15.73	27.11	32.75
GE (k cal/kg DM)	4323.9	4435.2	4017.1	3887.2
DE (k cal/kg DM)	3725.9	3720.3	3318.1	3188.5
NFC	62.94	54.49	32.37	28.51

MSSW, mango seeds soaked in water; **H**, Egyptian clover hay; **S**, wheat straw; **OM**, organic matter; CP, crude protein; **EE**, ether extract; **CF**, crude fiber; **NFE**, nitrogen free extract; **NDF**, neutral detergent fiber; **ADF**, acid detergent fiber; **ADL**, acid detergent lignin; **HC**, hemi-cellulose, **CEL**, cellulose; **GE**, gross energy (kcal/kg DM) was calculated according to Blaxter (1968). Each g CP= 5.65 kcal, g EE = 9.40, and g NFE and CF = 4.15 kcal; DE, Digestible energy (kcal/kg DM) was calculated according to DeBlas et al., (1992), using the following equation: (DE=GE × (0.867-0.0012ADF)) and **NFC**, non-fibrous carbohydrates was calculated according to Calsamiglia *et al.* (1995), using the following equation: NFC = 100 – {CP + EE + Ash + NDF}.

Composition and chemical analyses of concentrate feed mixture:

The chemical composition of tested concentrate feed mixtures (CFM) is presented Table 2. The MSSW was replaced by 0, 20, 40 and 60% of yellow corn content in a basal ration incorporated at 30% of ration formula. All tested CFM were isonitrogenous (14.40 - 14.78% CP) and isocaloric (4174.1 – 4195.1 kcal/kg DM). The present study showed significant (P<0.05) differences between different tested concentrate feed mixtures in terms of CF, NFE, NDF, ADF, ADL, CEL, HC and NFC contents. The content of the tested concentrate feed mixtures of OM, CP, EE, ash; GE and DE were approximately the same. The current study suggested that NDF was positively correlated with lignin but negatively correlated with NFC. Our results are in agreement with those reported by Jayanegara *et al.*, 2019. The highest CF, NDF, ADF, ADL, and CEL values were for T_{60}, while the lowest value was for T_0. No significant differences were found between T_0, T_{20}, T_{40} and T_{60} in OM, CP, EE and ash values. The present results are in agreement with those reported by Omer *et al*., 2019 and Admasu *et al*. 2020. Also, the present study indicated that replacing corn with mango seeds led to an increase in the value of ash (ranging from 8.48 to 11.96 g/100 g DM), EE (ranging from 2.4 to 3.52 g/100 g DM), and CF (ranging from 11.17 to 24.05 g/100 g DM) in the tested feed mixtures. In contrast, the value of nitrogen free extract decreased from 62.63 to 51.06 g/100 g DM. Our results showed that mango seeds are a good source of carbohydrate. These results agree with those reported by Diarra (2014) and Omar *et al.*, (2019).

Table 2. Chemical composition of the concentrate feed mixtures

Items %	T_0	T_{20}	T_{40}	T_{60}
OM	91.52	91.49	91.47	91.43
CP	14.78	14.66	14.53	14.40
EE	2.94	3.13	3.30	3.52
CF	11.17	12.37	13.59	14.78
NFE	62.63	61.33	60.05	58.73
Ash	8.48	8.51	8.53	8.57
NDF	34.76	35.85	37.29	38.95
ADF	12.92	16.30	19.38	23.16
ADL	4.47	5.11	7.35	8.42
HC	21.84	19.25	17.91	15.79
CEL	8.45	11.19	12.03	14.74
GE (kcal/kg DM)	4174.1	4181.1	4187.2	4195.1
DE (kcal/kg DM)	3554.3	3543.2	3532.9	3520.6
NFC	39.04	37.85	36.35	34.56

T_0=Concentrate mixture CP without replacement, **T_{20}, T_{40}** and **T_{60}** =Concentrate mixtures replaced with 20%, 40% and 60% of MSSW, respectively**, OM**, organic matter; **CP**, crude protein; **EE**, ether extract; **CF**, crude fiber; **NFE**, nitrogen free extract; **NDF**, neutral detergent fiber; **ADF**, acid detergent fiber; **ADL**, acid detergent lignin; **HC**, hemi-cellulose, **CEL**, cellulose; **GE**, gross energy (kcal/kg DM) was calculated according to Blaxter (1968). Each g **CP**= 5.65 kcal, g EE = 9.40, and g **NFE** and **CF** = 4.15 kcal; **DE**, Digestible energy (kcal/kg DM) was calculated according to DeBlas *et al.* (1992), using the following equation: (DE=GE × (0.867-0.0012ADF)) and **NFC**, non-fibrous carbohydrates was calculated according to Calsamiglia *et al.* (1995), using the following equation: NFC = 100 – {CP + EE + Ash + NDF}.

Chemical analyses of the concentrate feed mixture with roughage:

Data of Table 3 showed that all experimental total mixed rations (TMR) were isonitrogenous (14.52 and 8.15% CP in average for CH and WS group, respectively) and isocaloric (4059.53 and 3944.2 kcal/kg DM in average for CH and WS group, respectively). The present study showed significant ($P< 0.05$) differences between different tested TMRs in CF, ash, NDF, ADF and HC contents. The content of TMR of CF, ash, NDF, ADF, HC and CEL ranged from 16.74 to 28.05%, 10.87 to 13.67%, 37.97 to 46.70%, 21.15 to 25.87%, 16.24 to 21.27% and from 15.38 to 18.34%, respectively. The higher values of CF, ash, NDF, ADF and HC were in WS groups, while the values of NFE, GE, DE and NFC were higher in CH group. The values of OM, CP, CF, NFE, ash, GE and NFC in CH group are in agreement with those reported by Omer et al. 2019 who reported 89.79% OM, 14.52% CP, 17.04% CF, 53.8% NFE, 10.21% ash, 4177 kcal/kg DM and 30.72% NFC contents of boiled mango seed kernel (BMSK).

Table 3. Chemical composition of the concentrate feed mixtures with roughage (on a dry weight basis)

Items%	T_0+CH	T_0+WS	T_{20}+CH	T_{20}+WS	T_{40}+CH	T_{40}+WS	T_{60}+CH	T_{60}+WS
OM	89.13	86.37	87.92	86.36	87.91	86.35	87.89	86.33
CP	14.59	8.22	14.54	8.17	14.49	8.12	14.44	8.07
EE	2.39	2.15	2.46	2.22	2.53	2.29	2.62	2.38
CF	16.74	27.20	17.22	27.68	17.71	27.87	18.18	28.05
NFE	55.41	48.80	53.70	48.29	53.18	48.07	52.65	47.83
Ash	10.87	13.63	12.08	13.64	12.09	13.65	12.11	13.67
NDF	37.97	43.81	39.18	44.07	40.62	45.30	42.39	46.70
ADF	21.15	22.54	21.88	23.66	24.75	24.31	25.08	25.87
ADL	4.91	4.31	5.85	5.76	6.67	6.35	7.01	7.53
HC	16.82	21.27	17.30	20.41	15.87	20.99	17.31	20.83
CEL	16.24	18.23	16.03	17.90	18.08	17.96	18.07	18.34
GE (kcal/kg DM)	4053.2	3937.9	4057.6	3942.3	4061.3	3945.9	4066.0	3950.7
DE (kcal/kg DM)	3411.2	3302.9	3396.8	3295.6	3400.5	3306.0	3421.7	3322.6
NFC	31.94	31.47	32.45	31.51	31.19	29.88	30.89	30.46

T_0=Concentrate mixture CP without replacement, **T_{20}, T_{40}** and **T_{60}** =Concentrate mixtures replaced with 20%, 40% and 60% of MSSW, respectively**, OM**, organic matter; **CP**, crude protein; **EE**, ether extract; **CF**, crude fiber; **NFE**, nitrogen free extract; **NDF**, neutral detergent fiber; **ADF**, acid detergent fiber; **ADL**, acid detergent lignin; **HC**, hemi-cellulose, **CEL**, cellulose; **GE**, gross energy (kcal/kg DM) was calculated according to Blaxter (1968). Each g CP= 5.65 kcal, g EE = 9.40, and g NFE and CF = 4.15 kcal; DE, Digestible energy (kcal/kg DM) was calculated according to DeBlas *et al.* (1992), using the following equation: (DE=GE × (0.867-0.0012ADF)) and **NFC**, non-fibrous carbohydrates was calculated according to Calsamiglia *et al.* (1995), using the following equation: NFC = 100 – {CP + EE + Ash + NDF}.

In vitro gas production from concentrate feed mixtures (CFMs):

Data on gas production is presented in Table 4. The cumulative volume of gas increased with the progression of incubation times. Significant differences in gas production were evident between substrates for all the incubation times. Thus, gas productions at all incubation times were higher (P<0.05) with T_0 than with T_{20}, T_{40} and T_{60}. At 24 and 96 h incubation the total gas production for T_0 were significantly (P<0.05) higher than those of the other substrates. Gas production from protein fermentation is relatively small compared to carbohydrate fermentation, but when the major part of protein is part of the soluble fraction, the protein fermentation influenced gas production mainly in the initial hours of incubation (Cone and van Gelder, 1999). The current study showed that increasing the ether extract in the sample led to a decrease in total gas production (Tables 2 and 4). The results agree with the results of Adeyemi *et al*., (2015), where supplementation of various oils either did not affect or reduced the gas production. The addition of oil or cellulose to NDF residue resulted in a decrease or no effect on methane production and total gas production, respectively (Drehmel 2017). Kinetics of gas production obtained from the exponential model arepresented in Table 4. The gas production from a soluble fraction (a) of T_{20} was significantly (P<0.01) higher and greater for T_0 (P<0.05) than T_{40} and T_{60}. Gas production from an insoluble fraction (b) for T_0 and T_{40} was significantly (P<0.05) higher than T_{20} and T_{60}. However, the potential gas production (a+b) for T_{60} was significantly (P<0.05) lower than the other substrates. The current results indicated that replacing yellow corn with mango seeds led to decreased total gas production. This was linked to changes in the chemical composition. The current results confirmed that there is a significant (P<0.05) correlation between CP and gas production, and a negative relationship between CF, NDF, ADF and ADL contents and gas production (Tables 2 and 4). This result is in agreement with Karabulut *et al*. (2007).

Table 4. Cumulative gas produced and parameters of gas production at different incubation times of the concentrate feed mixtures

Items	12 h	24 h	48 h	72 h	96 h	a (ml)	b (ml)	c(ml/h)	a+b(ml)
T_0	28	48^a	55a	57^a	60^a	1.29^b	58.18^a	0.06^a	59.47^a
T_{20}	28	45^b	51^b	55^a	57^b	7.74^a	49.59^c	0.04^b	57.33^b
T_{40}	26	43^{bc}	52^b	55^a	57^b	0.78^c	56.26^b	0.05^{ab}	57.04^b
T_{60}	26	42^c	47^c	51^b	53^c	0.39^d	51.81^c	0.06^a	52.19^c

[a,b,c]Means within the same column with differing superscripts are significantly different.

Effect of type of roughage on gas production and estimated parameters:

The highest cumulative gas production was observed in T_0 + CH which, was followed by T_0 + WS and T_{40} + CH (Table 5). The cumulative gas production for T_{60} + WS was significantly low in comparison to other substrates. This is due to low NFE content for T_{60} + WS, which positively correlates with gas production. Gas production is the result of carbohydrates fermentation to short chain fatty acids (SCFA), gases, mainly CO_2 and CH_4, and microbial cells. Substantial changes in carbohydrate fractions were reflected by total gas produced (Deaville and Givens, 2001). Njidda and Nasiru, (2010) suggested that the gas production is a reflection of the effectiveness and extent of degradability of feed because forages that have a high ruminal degradability of dry matter tend to have high gas production. On the other hand, cell wall content (NDF and ADF) were negatively correlated with gas production and estimated parameters. This may tend to reduce the microbial activity through increasing the adverse environmental conditions as incubation time progress. This is in agreement with those reported by De Biover *et al*., (2005), who noted that gas production was negatively related with NDF and ADL content and positively with starch. Kinetics of gas production is dependent on the relative proportions of soluble and insoluble particles of the feed. The gas production from soluble fraction (a) was highest for T_{60} + WS (P < 0.01) and higher for T_{40}+CH and T_0 + WH (P<0.05) than the other substrates. The gas production values from insoluble fraction (b) ranged from 26.27 to 55.53 ml for T_{60}+WS and T_0+**CH**, respectively. However, the potential gas production (a+b) were highest for T_0+**CH** and T_0+WS (P < 0.01) and higher for T_{40}+BH (p<0.05) than T_{60}+WS. Gas production and estimated parameters (c, a, b and a + b) were positively correlated with CP, which is one of the limiting factors for microbial growth (Kamalak, 2005). The incubation pattern of high protein feedstuffs, protein being part of the soluble fraction, is usually characterized by initial fast fermentation and reached maximum after 20h of incubation and after 46h of incubation protein content is likely to be fully fermented (Cone and Van Gelder, 1999).

Table 5. Cumulative gas produced and parameters of gas production at different incubation times of the concentrate feed mixtures with roughage

Items	12 h	24 h	48 h	72 h	96 h	a (ml)	b (ml)	C(ml/h)	a+b(ml)
T_0+ CH	25^{ab}	42^{a}	50^{a}	54^{a}	57^{a}	1.11^{e}	55.53^{a}	0.05^{a}	56.65^{a}
T_0+WS	21^{cd}	40^{ab}	47^{b}	52^{ab}	56^{a}	3.16^{b}	52.83^{b}	0.04^{b}	55.99^{a}
T_{20}+CH	26^{a}	42^{a}	47^{b}	51^{b}	53^{b}	2.93^{c}	49.62^{cd}	0.05^{a}	52.55^{b}
T_{20}+WS	18^{e}	40^{ab}	42^{c}	49^{bc}	51^{b}	0.64^{f}	50.47^{c}	0.04^{b}	51.11^{bc}
T_{40}+CH	23^{b}	40^{ab}	48^{ab}	54^{a}	56^{a}	3.42^{b}	51.19^{bc}	0.03^{c}	54.61^{a}
T_{40}+WS	21^{c}	40^{ab}	48^{ab}	50^{b}	52^{b}	1.74^{d}	51.23^{b}	0.05^{a}	52.97^{b}
T_{60}+CH	23^{b}	39^{b}	43^{c}	48^{c}	51^{b}	2.00^{cd}	47.99^{d}	0.05^{a}	49.99^{c}
T_{60}+WS	19^{de}	28^{c}	28^{d}	31^{d}	35^{c}	6.47^{a}	26.27^{e}	0.05^{a}	32.74^{d}

^{a,b,c}Means within the same column with differing superscripts are significantly different.

Energy contents, total digestible nutrients, organic matter digestibility, short chain fatty acids, microbial protein (MP) and methane production for different CFMs:

The predicted metabolizable energy (ME, MJ/kg DM), net energy lactation (NEL, MJ/kg DM), total digestible nutrients (%), organic matter digestibility(%), short chain fatty acids, microbial protein (MP) and methane production for different CFMs are presented in Table 6. Our data indicates that the values of ME and NEL ranged from 7.78 to 8.72 and from 4.55 to 5.24 MJ/kg DM, respectively. The ME and NEL were higher ($P < 0.05$) for T_0 and T_{20} than for T_{60}. There was a negative correlation between metabolizable energy and fiber content and a positive correlation to crude protein content, which is in agreement with the findings of Tolera *et al.* (1997). Fiber is known in affecting ME content of the substrates due to the fact that the digestibility of protein will decrease when fiber intake increases, and that will reduce the ME content of the diet (Baer, *et al*, 1997). There was a positive correlation between ME calculated from *in vitro* gas production together with CP and fat content with ME value of conventional feeds measured in vivo (Menke and Steingass, 1988). Chenost *et al*. (1997) concluded that the prediction of ME is more accurate when based on gas and chemical constituents measurements as compared to calculations based on chemical constituents only. The values of TDN and OMD ranged from 51.88 to 56.89 and from 59.26 to 64.76 %, respectively. The lower values of TDN and OMD were for T_{60}. In general, indigestible substances like lignin, acid insoluble ash will interfere with the digestibility of other useful nutrients and, hence, feeds with high lignin and/or acid insoluble ash have low TDN and OMD values. Mokoboki *et al*. (2019) showed that there was a negative correlation between acid detergent fiber content and dry matter degradability. This result was in agreement with Kamalak *et al*. (2005), while in contrast to the results reported by Repetto *et al*. (2003) showed that ADF has the closest relationship with DMD. There are a significant correlation between *in vitro* gas measurement and in vivo digestibility (Menke and Steingass, 1988). SCFA and microbial proteins ranged from 92.82 to 106.14 mM and from 71.48 to 78.11 g/kg OMD, respectively. Total SCFA concentration and true digestible organic matter (TDOM) were positively correlated with CP, EE and non-fiber carbohydrate (NFC) content, and negatively with NDF and ADF content (Pal *et al* 2015). The present dataare in agreement with the results of Ma *et al*. (2014) who found that high dietary level NFC proved that high dietary level NFC could improve ruminal concentration of total VFA. Microbial proteins were significantly ($P<0.05$) higher for T_0 and T_{20} than T_{60}. CH_4 ranged from 22.2 to 29.6% Table 6. The higher value of methane was for T_{60} than the other substrates. The present results agree with the results of Drehmel, 2017 who suggested that methane per unit of digested NDF tended to decrease with increasing hemicellulose concentration. Methane produced per gram of digested cellulose is 5 times greater than methane produced per gram of digested non-fiber carbohydrates (i.e. starch) (Moe and Tyrrel1, 1979). Holter and Young (1992) support these estimates and further suggest the cellulose could be the fiber fraction that contributes most to methane production in lactating dairy cows consuming mixed forage-concentrate diets.

Table 6. Metabolizable energy (ME), net energy lactation (NEL), total digestible nutrients (TDN), organic matter digestibility (OMD), short chain fatty acids (SCFA) microbial protein (MP) and methane for different concentrate feed mixtures, *in vitro*

Items	ME (MJ/kg DM)	NEL (MJ/kg DM)	TDN %	OMD %	SCFA (mM)	MP (g/kg OMD)	CH4 %
T_0	8.72^{a}	5.24^{a}	56.89^{a}	64.76^{a}	106.14^{a}	78.11^{a}	23.7^{b}
T_{20}	8.25^{b}	4.89^{b}	54.37^{b}	62.04^{b}	99.48^{b}	74.83^{b}	22.2^{b}
T_{40}	7.94^{bc}	4.66^{bc}	52.70^{bc}	60.20^{bc}	95.04^{bc}	72.62^{bc}	22.8^{b}
T_{60}	7.78^{c}	4.55^{c}	51.88^{c}	59.26^{c}	92.82^{c}	71.48^{c}	29.6^{a}

Mean separation with the same letter in a column are not significantly different at $P<0.05$.

Effect of roughage type on energy contents, total digestible nutrients, organic matter digestibility, short chain fatty acids, microbial protein (MP) and methane production:

The ME, NEL, TDN, OMD, SCFA, MP and CH_4 of various TMRs has been presented in Table-7. The ME and NEL ranged from 6.05 to 7.74 and from 3.26 to 4.52 MJ/kg DM, respectively. Lower ME value (6.05 MJ/kg DM) and NEL value (3.26 MJ/kg DM) were found in T_{60}+SW. such sample is high in CF, NDF, ADF and ADL and low in crude protein and fermentable carbohydrates (Table 3). Garg*et al*. (2012) suggested that ME values were very low in the feedstuffs having high fiber and low protein contents. Fiber is known to affect ME content of the substrates due to the fact that the digestibility of protein will decrease when fiber intake increases and that will decrease the ME content of the diet (Baer, *et al*, 1997). The TDN and OMD ranged from 42.55 to 51.65 % and from 44.29 to 59.49%, respectively. Lower TDN and OMD values were found in T_{60}+WS. Total digestible nutrients represent utilizable energy content of any feedstuff. There was a positive correlation between ME, NEL and TDN and digestibility. The TDN in forage and concentrate were negatively correlated with NDF (P<0.001) and lignin (P<0.05), but positively correlated with NFC and EE contents (both at P<0.001), Jayanegara, *et al*. 2019), supports the results of the current study.The *in vitro* digestibility and gas production parameters were significantly correlated with chemical composition of shrubs (Nasser, 2012). Gürbüz, *et el*, 2008 showed that there were significant negative correlations between content of condensed tannin and gas production, OMD and ME. The SCFA and MP ranged from 61.74 to 92.82 mM and 53.43 to 71.76 g/kg OMD, respectively. SCFA are the primary source of metabolizable energy in ruminants; thus, their production in the rumen would be essentialparameter for feed evaluation (Moran, 2005). SCFA concentration was higher in T_0+CH, and T_{20}+CH and lower in T_{60}+WS (Table 7). Microbial activity was negatively affected by the bound condensed tannin (BCT) content of leaves, possibly due to reduced organic matter available for micro-organisms (Kamalak *et al.,* 2004).Methane produced per gram of digested cellulose is 3 times greater than the methane produced per gram of digested hemicellulose (Moe and Tyrrel, 1979).

Table 7. Effect of type of roughage on metabolizable energy (ME), net energy lactation (NEL), total digestible nutrients (TDN), organic matter digestibility (OMD), short chain fatty acids (SCFA) microbial protein (MP) and methane for different concentrate feed mixtures with roughage, *in vitro*

Items	ME (MJ/kg DM)	NEL (MJ/kg DM)	TDN %	OMD %	SCFA (mM)	MP (g/kg OMD)	CH_4 %
T_0 +CH	7.74^{a}	4.52^{a}	51.65^{a}	59.49^{a}	92.82^{a}	71.76^{a}	22.8^{cd}
T_0+WS	7.68^{ab}	4.41^{b}	51.31^{ab}	55.03^{cd}	88.38^{ab}	66.38^{c}	29.6^{b}
T_{20}+CH	7.73^{a}	4.52^{a}	51.61^{a}	59.55^{a}	92.82^{a}	71.83^{a}	22.0^{d}
T_{20}+WS	7.68^{ab}	4.41^{b}	51.30^{abc}	55.00^{c}	88.38^{ab}	66.35^{c}	27.5^{bc}
T_{40}+CH	7.42^{bc}	4.29^{c}	49.93^{bc}	57.75^{b}	88.38^{ab}	69.66^{b}	23.2^{cd}
T_{40}+WS	7.68^{ab}	4.41^{b}	51.30^{ab}	54.98^{c}	88.38^{ab}	66.32^{c}	30.9^{ab}
T_{60}+CH	7.26^{c}	4.17^{d}	49.09^{c}	56.84^{bc}	86.16^{b}	68.56^{bc}	20.8^{d}
T_{60}+WS	6.05^{d}	3.26^{e}	42.55^{d}	44.29^{d}	61.74^{c}	53.43^{d}	34.3^{a}

Mean separation with the same letter in a column are not significantly different at p<0.05**.**

CONCLUSION

Mango seed has an alternative nutritional attribute that is useful for using in ruminants feed. Consequently, mango seeds are the best promising solution as an economic for energy. We need more *and in vivo*studies to determine mango seed's effect on animal performance, quality of production (meat and milk), and methane production.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ANIMAL WELFARE STATEMENT

All procedures and experimental protocols were carried out meeting the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

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تأثير الإستبدال الجزئي للذرة الصفراء ببذور المانجو على التحلل في الكرش ومحتوى الطاقة وإنبعاث الميثان معملياً

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تعتبر بذور المانجو، غير المستغلة بواسطة الإنسان، مكونًا غذائيًا محتملاً ومصدرًا جيدًا للكربوهيدرات القابلة للذوبان والبروتين و نسبة الدهون فيها أعلى من نسبتها في الذرة. أجريت هذه الدراسة بهدف فحص تأثير استبدال الذرة الصفراء ببذور المانجو المنقوعة في الماء (MSSW) في العلائق المركزة والعلائق التجريبية على إنتاج الغاز و تخمرات الكرش معملياً. تم تكوين أربعة مخاليط مركزة تتكون من ٠ ، ٢٠ ، ٤٠ و ٦٠٪ MSSW ، يشار إليها بـ T_0، T_{20}، T_{40} و T_{60} ، على التوالي. تتكون العلائق التجريبية من المخاليط المركزة السابقة مع دريس البرسيم (CH) و / أو تبن القمح (WS). العليقة الأولى (T_0 + CH)، العليقة الثانية (T_0 + WS)، العليقة الثالثة (T_{20} + CH)، العليقة الرابعة (T_{20} + WS)، العليقة الخامسة(T_{40} + CH)، العليقة السادسة(T_{40} + WS)، العليقة السابعة(T_{60} + CH) و العليقة الثامنة (T_{60} + WS)، على التوالي. تم تقييم آثار الاستبدال عن طريق تحضين العلائق المختبرة في سائل الكرش المخزن باستخدام تقنية الغاز معملياً. تم حساب الطاقة الميتابوليزمية ، الطاقة الصافية ، وإجمالي العناصر الغذائية القابلة للهضم ، وقابلية هضم المواد العضوية (OMD) من إنتاج الغاز بعد فترة الحضانة لمدة ٢٤ ساعة. تم حساب البروتين الميكروبي من OMD. كان انبعاث الميثان مرتفعًا في T60 و WS + T60 مقارنةً بالعلائق الأخرى المختبرة.

الخلاصة، تتميزبذورالمانجوبخاصية غذائية بديلة مفيدة للاستخدام في علف المجترات. وبالتالي،فإن بذورالمانجوهي أفضل حل واحد كحل اقتصادي للطاقة. نحتاج إلى المزيد من الدراسات في الجسم الحي لتحديد تأثير بذورالمانجو على أداءالحيوان،وجودة الإنتاج (اللحوم والحليب)، وإنتاج الميثان.