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The Effect of using Deciduous Mango Leaves and Trimmings of Mango Wasted, Containing Tannin, on *in Vitro* Rumen Methanogenesis and Fermentation Parameters

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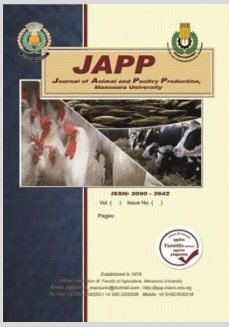

ABSTRACT

Tannins is the novel feed strategy that can mitigate enteric methane emissions. This study characterized the response of in vitro rumen methanogenesis and the ruminal fermentation of Mango tree by- products (deciduous mango leaves (DML) and Waste Trimmings of Mango Trees (WTM)) rich in tannins. Results of Anti-nutritional factor founded that both of DML and WTM showed higher value of tannins (2.0 and 2.4 mg/100g) respectively. The highest significant GP, gas production structure fiber (GPSF) and gas production non structure fiber (GPSNF) were observed in DML and recorded 20.44, 10.04 and 34.49 (ml/g DM), respectively. The First laboratory experimental study results exhibited highly significant differences in ruminal fermentation characteristics across control diet and diets with DML. The highest significant GP, GPSF and GPSNF were observed in control diet which recorded 49.19 (ml/200mg DM), 14.26 (ml/g DM), and 43.45 (ml/g DM), respectively. Also, ME (MJ/Kg DM), NE, DCPL (g/day), DOMI (g/day), Microbial protein (g/DOM kg), and GED (g/Kg DDM) were significantly higher with control diet. The obtained results of the second laboratory experiment showed significant over than the control diet in GPSF, GPNSF, SCFA, energy content, DOM, ME (MJ/Kg DM) , NE, DCPL (g/day), DOMI (g/day), microbial protein (g/DOM kg), and GED (g/Kg DDM) in comparison with 5% and 10% from WTM as a replacement of wheat bran. The results of the in vitro methanogenesis study found that Mango tree by- products containing tannins have the ability to inhibit methanogenesis.

Keywords: Deciduous mango leaves, Waste Trim Mango Trees, Tannins, Methane.

INTRODUCTION

A global attention was devoted to methane emission from ruminants due to producing the greenhouse gases effect and the global heating climate change (Martin *et al*., 2010 and Hixson JL *et al.*, 2018). Methane is one of trace and strong gases because of its global warming possibility, exceeded 25 times over Co2, it is considering the second atmospheric greenhouse gas, behind CO2 (Forster *et al*., 2007 and IPCC, 2001). Globally, agricultural sector contributed more than 50% of methane emissions, particularly from livestock rumen fermentation. Methane emitted in the rumen by rumen microbes after fermentation of feed to get rid of hydrogen. McMichael *et al*., (2007) reported that 86 million mega grams of methane year-1 were produce from domesticated ruminants, like goats, sheep, and cattle. Johnson and Johnson, (1995) showed that consistence of Methane led to a loss about 8 to 10 % of gross energy intake for the animal. Thus, decreasing methane formation in ruminants would enhance their feed efficiency. Many investigators (Beauchemin et al., 2008; Cottle et al., 2011; Eckard et al., 2010; Martin et al., 2010) are in harmony with applied various strategies to decrease enteric methane emission from ruminants.

Stumm *et al*., (1982) demonstrated that protozoa supply 10 to 20% of rumen methanogens also; manufacture butyrate and acetate as substrates for methanogenesis. Disposal of protozoa (defaunation) applied to prevent methane release. Consequence, it is done in a dietary way i.e. decrease pH there is a great harmful of causing ruminal acidosis (Kreuzer et al., 1986). Use of artificial chemicals (e.g. calcium peroxide or copper sulphate) or natural compounds (e.g. steroidal hormones, non-protein amino acids and vitamin A), prevent of protozoa propagation (Hegarty 1999). Also, he reported that absence of rumen protozoa led to decrease of methane release on average by 13% across a range of diets. Moreover, Eugène et al., (2008) indicated that defaunated animals able to reduce digestibility of total nutrient.

Plant tannins as rumen modifiers are look superior over antibiotic-based modifiers or chemicals. Therefore, these substances are natural products and environmentally friendly. Also, plant tannins have a better approved for their feed safety. Plants involving tannins eliminate or inhibit protozoa from the rumen consequence, reduce ammonia and methane release. Patra, (2010) demonstrated that focus on digestibility and rumen fermentation should be given, because the supplementation of tannins predominating decreases digestibility [e.g. of acid detergent fiber (ADF) and neutral detergent fibre (NDF)] tends to lowering total VFA production. The gas release way *in vitro* has been spread used for estimating of methanogenesis and the nutritive value to various types of plants (Sallam, 2005, and Vitti *et al*., 2005) and various classes of feedstuffs (Sallam *et al*., 2008). The objective of this research was to discuss the impact of deciduous mango leaves and Wasted Trimmings of Mango Trees involving tannin on *in vitro*

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methanogenesis and fermentation parameters on the degradation of nutrients and the methane synthesis and on microbial protein and the production of total gas Additionally, concentrations of tannin should be defined for a maximal methane reduction without adverse effects on fermentation.

MATERIALS AND METHODS

Experimental site

This study was carried out at the laboratory of chemical analysis, antinutritional factor and Total nutrient digestibility, Regional center for food and feed, Agriculture Research Centre (ARC), Ministry of Agriculture. Giza. Egypt. It was conducted in August 2019.

Source of mango by-product

Deciduous mango leaves and Trimmings of Mango trees were harvested from the mango plants, which are abound in private farms in Ismailia Governorate. Egypt.

Experimental diets

Chemical analysis, anti-nutritional factor, *in vitro* methanogenesis and fermentation parameters were conducted on different samples of deciduous mango leaves (DML) and Wasted Trimmings of Mango (WTM).

The *in vitro* experimental method and techniques included two laboratory experimental studies. The first experiment was *in-vitro* methanogenesis and fermentation parameters to examine the effect of replacing DML from 20% and 40% of wheat bran protein on the production of methane, total gas, rumen fermentation parameters and the microbial protein synthesis. While the second laboratory experiment was in-*vitro* methanogenesis and fermentation parameters to examine the effect of replacing WTM from 20% and 40% of wheat bran protein on the production of methane, total gas, rumen fermentation parameters as well as microbial protein synthesis.

Chemical analysis

DML and WTM were air dried overnight in air drying oven on 60C^0, collected samples were milled and passes through a 1 mm sieve for *in vitro* gas production procedure and chemical analysis. to determine dry matter (DM), samples were drying at 105°C 3hr. and ash were determined after exposing samples to 600°C for 2 hr. at muffle oven. kjeldahl method was applied to measured Nitrogen (N) content. then Crude protein (CP) was estimated as (N*6.25), crude fiber (CF) and Ether extract (EE) was determined according to the method AOAC (1995).

NDF-ADF= Hemicellulose

ADF-ADL= Cellulose

Alkaloid content was determined according to method as described by Harbone (1973) using the gravimetric. The saponin, oxalate and tannin were estimated according to method of Pearson (1976). Phytate content determined using the method as described by Oberleas (1973).

First experimental study

Rations were formulated to be in iso-nitrogenous iso- caloric rations. T1 the control diet (concentrate feed mixture). T2 the control diet supplemented with 20% from deciduous mango leaves (DML) as a replacement from wheat bran protein. T3 the control diet supplemented with 40% from DML as a replacement from wheat bran protein. Ingredient diets percentage are presented in Table (2).

Table 1. Chemical analysis of dried mango leaves deciduous Waste Trim Mango Trees and wheat bean (on DM basis)

Nutrients	deciduous mango leaves %	Waste Trim Mango Trees %	Wheat bran %
Dry matter	92.66	93.80	92.2
CP	6.72	8.19	13
EE	4.10	4.93	3.9
Crude fiber	24.68	29.88	12
Ash	10.45	4.51	6.2
Nitrogen free extract	54.05	52.49	64.9
NDF	35.46	44.23	30.07
ADF	30.98	35.32	25.91
ADL	12.98	15.97	5.06
Hemicellulose	4.48	8.91	4.16
Cellulose	18	19.53	20.85

CP: Crude protein; EE: Ether extract; Crude fiber: CF; NFE: Nitrogen free extract;

NDF: Neutral detergent fiber; ADF: Acid detergent fiber; ADL: Acid detergent lignin

Table 2.Experimental concentrate mixtures and chemical analysis of diets containing deciduous mango leaves substitution of 20% and 40% from wheat bran protein (on DM basis) .

Ingredients	experimental concentrate mixtures(kg/ton)		
	T1	T2 (20% DML)	T3 (40% DML)
Yellow corn	450	450	450
Soybean meal 44%	180	180	180
Wheat bran	300	288.4	276.8
Molasses	40	40	40
Calcium carbonate	20	20	20
Sodium chloride (Na Cl)	10	10	10
Deciduous mango leaves	0	11.6	23.2
Calculated nutrients %			
CP	16	16	16
EE	3.15	3.16	3.23
CF	5.95	6.61	7.33
Ash	4.65	4.21	4.31

Second experimental study:

Rations were formulated to be in iso-nitrogenous iso- caloric rations. T1 the control diet (concentrate feed mixture). T2 control diet supplemented with 20% of Waste Trim Mango Trees (WTM) as a replacement to wheat bran protein. T3 control diet supplemented with 40% from WTM as a replacement for wheat bran protein. ingredient diets percentage are presented in Table (3).

Table 3. Experimental concentrate mixtures and chemical analysis of diets containing Waste Trim Mango Trees substitution of 20% and 40% from wheat bran protein (on DM basis)

Ingredients	Experimental concentrate mixtures(kg/ton)		
	T1	T2 (20% WTM)	T3 (40% WTM)
Yellow corn	450	450	450
Soybean meal 44%	180	180	180
Wheat bran	300	290.48	280.96
Molasses	40	40	40
Calcium carbonate	20	20	20
Sodium chloride (Na Cl)	10	10	10
Waste Trim Mango	0	9.52	19.04
Calculated nutrients %			
CP	16	16	16
EE	3.15	3.19	3.22
CF	5.95	6.51	7.08
Ash	4.65	4.16	4.28

Gas production In vitro

Rumen fluid was collected from three fistulated Rahmani rams fed twice daily at the maintenance level with a basal diet containing concentrate (40%) and alfalfa hay (60%).

Incubation process according protocol of Menke *et al.* (1979) was applied on the samples *in vitro* with rumen fluid in calibrated glass syringes. Reactions were carried out in triplicate into calibrated glass syringes of 100 ml using 200 Milligram dried samples and prewar med at 39°C. Then, add 30 ml rumen fluid- buffer mixture into each syringe and syringes put in a water bath at 39°C to be incubated. Then, gas production was recorded after eight incubation time following as 0, 2, 4, 6, 8, 12, 24 and 48 hours. A blank incubation was undertaken to correct reading of total gas. According to model of Orskov (1998) Cumulative gas production data were fitted.

Digestible dry matter (DDM), Digestible Organic matter (DOM), Short chain fatty acids (SCFA), Metabolizable Energy (ME), net energy lactation (NEL) ,DCPL (g/day), DOMI (g/day) , GED (g/Kg DDM), GED (g/Kg DOM) and TDN (%) values were estimated using equations as below according to Menke et al. (1979). Where gas production structure fiber (GPSF) (ml/g DM), gas production non-structure fiber (GPNSF) (ml/g DM), gas production (GP) after 24h. incubation (ml.200mg-1 DM) was estimated as described by Orskov (1998).

Equations

In vitro digestibility crude protein intake (DCPIinv) (G/day) =(-203.242+(14.797*GP24+ 6.249*GP48).

In vitro digestibility organic matter intake (DOMIinv) (g/ day) =(-1763.07+42.5*GP24) + 13.52*GP48). (van Gelder et al., 2005)

Short chain fatty acid (SCFAinv) (mmol /ml gas) =(0.0239 *GP+0.0601).

Net Gas production (ml/200mg DM) Gas production structure fraction (GPSF) (ml/g DM) = (GP3h-5.5) *0.99-3.

Gas production non- structure fraction GPNSF (ml/g DM) = (1.02*(GP24h -5.5) –(GP3h-5.5) +2).

Net energy (NEinv) (M cal./Lb.) = (2.2+(0.0272*gas) +(0.057*cp) +(0.149*EE)/14.64.(Menke and steingass, 1988)

Metabolic energy (MEinv) (MJ/kg DM) = 2.04+0.1448*GP+0.0036 CP+0.0243EE.

Net energy lactation (NELinv) (MJ/Kg DM) = 0.08+0.1101GP+0.0022CP+0.0161.

MP = Microbial protein g/kg (DOM)= 120*DOM/100. (Czerkawski, 1986)

Organic matter digestibility OMDinv, % = 14.88+ 0.889 GP + 0.45 CP + 0.065(Nousiainen et al., 2009)

Growth energy digestibility GED (g/kg) = −11.3 ±14.78 + 0.977 ±0.021× OMD

Growth energy digestibility GED (g/kg) = −12.7 ±18.4 + 1.00 ±0.027× DMD

where

DMD = DM digestibility.

TDN was calculated from ME value as per the equation of NRC (1989).

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

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

Statistical analysis

The collected data obtained were statistically analyzed using statistical analysis Software (SAS 1996) general linear model procedure. One-way analysis was performed where, complete randomize design (CRD) were applied. Then, Duncan's multiple tests (1955) were followed to compare among means.

The following model was used:

$$Y_{ij} = \mu + T_i + e_{ij},$$

Where:

Yij = Individual observation,

μ = overall mean, Ti = effect of treatment eij = random error.

RESULTS AND DISCUSSION

1- Chemical analysis of the deciduous mango leaves and Wasted Trimmings of Mango Trees

Results of chemical composition for the DML, WTM Trees and wheat bran are showed in Table (1). Results given that wheat bran recorded the elevated values in crude protein (13%), and Nitrogen free extract (64.9%). On the other hand, higher Ash was recorded by DML. Abel IO *et al*., 2018 found the proximate composition of dried mango leaf was higher values in crude protein (17.20%), Nitrogen free extract (43.34%), ash (11.25%), ether extract (5.81%) and crude fiber (22.40%) are higher. Waste Trim Mango Trees were found to be slightly higher in NDF, ADF, ADL, Hemicellulose and Cellulose.

2- Anti-nutritional factor content (mg/100 g) of deciduous mango leaves and Waste Trim Mango Trees

Results of Anti-nutritional factor for the DML, WTM Trees as a two natural sources of tannin and wheat bran are listed in Table (4). The results founded the same values of Alkaloid and Saponin on DML and WTM Trees (1.49 mg/100mg), Phytate concentration (3.68 mg/100g) is higher in wheat bran than WTM and DML. The WTM showed a higher value in Oxalate (1.66 mg/100g) and tannin (2.4 mg/100g) than obtained from DML and wheat bran. The values for Anti-nutritional factors obtained in this analysis correspond to the values reported by (Abel IO *et al*., 2018).

Table 4. Anti-nutritional factors content of dried mango leaves deciduous, waste trim mango trees and wheat bran. (mg/100 g)

Anti-nutrients	deciduous mango leaves (mg/100g)	Waste Trim Mango Trees (mg/100g)	Wheat bran (mg/100g)
Tannins	2.0	2.4	0.06
Alkaloid	0.02	0.01	0.02
Phytate	1.94	1.49	3.68
Saponin	0.08	0.06	0.02
Oxalate	1.42	1.66	0.32

3- *In Vitro* rumen methanogenesis and fermentation parameters of different samples of deciduous mango leaves and Waste Trim Mango Trees.

The effect of different samples of DML and WTM Trees on gas production and fermentation parameters presented in Table (5). The results recorded significant (P<0.01) differences in the ruminal fermentation parameters between DML and WTM Trees. The elevated significant in gas production, GPSF and GPSNF are noticed in DML were founded 20.44 (ml/200mg DM), 10.04 and 34.49 (ml/g DM), respectively. The average DDM and DOM of DML noticed to be the highest while the lowest was founded in WTM Trees. These results were due to the significant (P<0.01) increase in the ruminal fermentation parameters of DML. The parameters of GP showed similar trend with DOM. *In- vitro* SCFA was significantly increased 44.97 mmol/ml gases for DML than with 28.89 mmol/ml gas .

Also, ME and NEL were significantly high with DML. The highly significant Microbial protein was 80.60 (g/DOM kg) with DML and lowest with WTM Trees. These obtained results related to highly tannins contained on WTM Trees. Mueller-Harvey (2006) summarized the benefits of tannins in ruminant feeding. One of this benefit is the effect on digestion of protein. Tannins can reduce the amount of degraded protein in rumen and boost the by passing of protein to lower gut (McSweeney *et al*., 2001). Jansman, (1993) reported that protein and DM digestibility negatively influenced by tannins content.

Table 5. *In- vitro* rumen methanogenesis, nutrient degradability and rumen fermentation parameters to different samples of deciduous Mango leaves and Waste Trim Mango Trees .

Items	Dried Mango leaves deciduous	Waste Trim Mango Trees.	SE
GP (ml/200mg DM)	20.44[a]	13.20[b]	0.280
GPSF (mL/g DM)	10.04 [a]	5.09 [b]	0.521
GPNSF (ml/g DM)	34.49 [a]	29.02 [b]	0.981
DDM %	33.63 [a]	28.08 [b]	1.079
DOM %	66.22 [a]	60.27 [b]	0.471
DOMI (g/day)	820.27 [a]	265.23 [b]	1.772
DCPL (g/day)	141.07 [a]	64.69 [b]	8.824
ME (MJ/Kg DM)	8.15 [a]	7.00 [b]	0.063
NE (MJ/kg DM)	2.42 [a]	1.61 [b]	0.031
NEL (MJ/Kg DM)	3.99 [a]	3.03 [b]	0.179
SCFA (mml/ml gas)	44.97 [a]	28.89 [b]	0.625
SCFA (µmol/g DM)	0.43 [a]	0.26 [b]	0.006
Microbial protein (g/DOM kg)	80.60 [a]	72.70 [b]	0.504
Methane (ML/200mgDM)	5.15 [a]	4.72 [b]	0.001

a, b, c: within rows, values followed by the same letter are not significantly different (P=0.05).

The lowest significant value of Methane (ML/200mgDM) was recorded with the WTM Trees compared with DML, due to WTM Trees highly content of tannins. Bhatta *et al*., (2009) reported that tannins have been increasingly investigated reduce the methane emission of ruminants, via eliminating the rumen protozoa. Either this might be due to the high content of fiber fractions ((NDF), (ADF) and (ADL)), which were affected inversely on gas production. (Hovell *et al.,* 1986). Gas production and digestibility were negatively correlated to Neutral detergent fiber, lignin and poly phenolic compound (Ammar *et al.,* 2005). Condensed tannins interfere with microflora and attachment to feed particles and show the inverse effects on the microbial population inhibiting ruminal fermentation. (Patra, 2010) reported that *In vitro* trials are useful to show the different of natural tannin sources for their effects on methane and distinguish the most effective ones. higher attention should be given to rumen fermentation parameters and digestibility because the adding of tannins often decreases digestibility and rumen total volatile fatty acid production.

Gas production (GP), gas production structure fiber (GPSF) (ml/g DM), gas production non-structure fiber (GPNSF) (ml/g DM). Digestible dry matter (DDM), Digestible organic matter (DOM), digestible organic matter intake (DOMI), digestible crude protein lactation (DCPL), metabolizable energy (ME`), Net energy (NE), net energy lactation (NEL) , short chain fatty acid (SCFA).

4- ***In- vitro* rumen methanogenesis, nutrient degradability and rumen fermentation parameters for the first experimental concentrate feed mixtures consisting different levels of deciduous mango leaves.**

The predicted rumen melanogenesis, gas production GP (ml/200mg DM), gas production from soluble fractions (GPSF, ml/g DM) and gas production from non-soluble fractions (GPNSF, ml/g DM). Nutrient degradability and rumen fermentation parameters, short chain fatty acids (SCFA, mml/ml gas), net energy (NE, MJ/kg DM), metabolizable energy (ME, MJ/kg DM), organic matter digestibility (DOM)%, microbial protein (MP, g/kg DOM), NEL (MJ/Kg DM), DCPL (g/day), DOMI (g/day), GED (g/Kg DDM), GED (g/Kg DOM) and TDN (%) are illustrated in Table 6. and Figure 1. The obtained results recorded significant (*P<0.01*) differences in the ruminal fermentation parameters across control diet and diets supplemented with DML as a replacement of wheat bran protein. The highest significant value Gp, GPSF and GPSNF were with control diet 49.19 (ml/200mg DM), 14.26 (ml/g DM), 43.45 (ml/g DM), respectively, and the lowest values were with diet contained 40% of DML. These results due to the gas production was inversely affected by tannins, NDF and lignin. The lower *in vitro* degradation of organic matter in diets contained DML may be due to the inverse relationships between NDF, ADL and tannins with digestibility (Ammar *et al*., 2005). tannins interfere with microflora and attachment to feed particles and show the inverse effects on the microbial population inhibiting ruminal fermentation. Min and Wright (2014) reported that tannins can modify the ruminal microbiome which associated with decreased ruminal degradation of proteins, decrees of methanogenesis and inhibition of biohydrogenation of unsaturated fatty acids. The mean of DDM and DOM of diet with 40% from DML (T3) appeared to be the lowest while the higher was observed in control diet (T1) and DML substitution of 20% from wheat bran protein (T2). The parameters of GP recorded similar trend with DOM that were highest with control diet. This agrees with the results obtained by Haddi, *et al.* (2009) who founded that there was significant negative correlation between NDF, ADF and GP. The negative effect of cell wall content on GP may be due to the decreasing of the microbial activity through increasing the adverse environmental conditions like incubation time. *In- vitro* SCFA was significantly affected and extended between 108.78 to 109.88 mmol/ml gases with control diet and diet supplemented with 20% from DML (T2). Also, ME (MJ/Kg DM), NE, DCPL (g/day), DOMI (g/day), Microbial protein (g/DOM kg), and GED (g/Kg DDM) were significantly higher with control diet. No significant differences amongst all experimental diets were found on TDN (%). These results match with Fagundes *et al*. (2020) who founded that Methanogenic bacteria and protozoa populations, and methane production were reduced ($P < 0.05$) by condensed tannin (CT)- rich legumes *in vitro* methane emissions and rumen microbiota in beef cattle. Piñeiro-Vázquez AT *et al*. (2018) quebracho tannins extract supplementation at 2 or 3% of dry matter can decreasing methane production up to

29 and 41%, respectively, without decreasing feed intake and nutrients digestibility on the *In vivo* study.

Table 6. *In vitro* rumen methanogenesis, nutrient degradability and fermentation parameters of the first experimental concentrate feed mixtures consisting different levels of deciduous mango leaves.

Items	Experimental concentrate feed mixtures			SE
	T1	T2	T3	
DDM %	74.05[a]	64.62[b]	56.14[c]	0.363
DOM %	72.26[a]	69.85[ab]	68.63[b]	1.032
GP (ml/200mg DM)	49.19 [a]	49.69[a]	43.95 [b]	1.236
SCFA (mml/ml gas)	108.78[a]	109.88[a]	97.14[b]	2.744
GPSF (ml/g DM)	14.26[a]	10.45[b]	9.22[c]	0.257
GPNSF (ml/g DM)	43.45[a]	31.09[b]	27.13[c]	0.916
ME (MJ/Kg DM)	9.19[a]	9.18[a]	9.03[a]	0.323
NEL (MJ/Kg DM)	4.94[b]	5.64[a]	5.06[ab]	0.188
NE (MJ/Kg DM)	3.9[a]	3.60[b]	3.58[b]	0.039
DCPL (g/day)	269.8[a]	115.64[b]	97.45[c]	1.749
DOMI (g/day)	2080.42[a]	1052.32[b]	883.82[b]	5.862
Microbial protein (g/DOM kg)	87.16[a]	84.26[ab]	82.78[b]	1.246
GED (g/Kg DMD)	59.29[a]	56.95[ab]	55.74[b]	1.009
GED (g/Kg OMD)	61.35[a]	51.92[b]	43.44[c]	0.363
TDN (%)	59.47[a]	59.41[a]	58.58[a]	1.735

a, b, c: within rows, values followed by the same letter are not significantly different (P=0.05). a,b,c, mean within some rows with differing superscript are significantly differ (P<0.05). T1 the control diet (concentrate feed mixture). T2 the control diet supplemented with 20% from deciduous mango leaves as a replacement of wheat bran protein. T3 the control diet supplemented with 40% from deciduous mango leaves as a replacement of wheat bran protein.

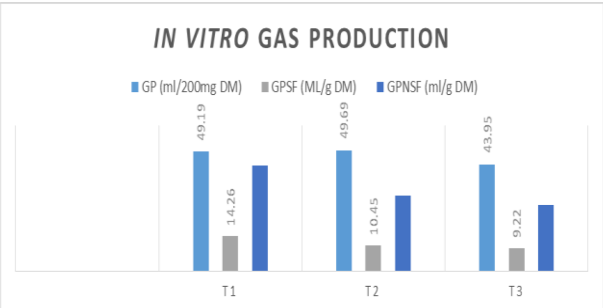

Figure 1. *In vitro* rumen methanogenesis of the first experimental concentrate feed mixtures consisting different levels of deciduous mango leaves

5-*In- vitro* rumen methanogenesis, nutrient degradability and rumen fermentation parameters for the second experimental concentrate feed mixtures consisting different levels of Waste Trim Mango

The predicted rumen melanogenesis, gas production GP (ml/200mg DM), gas production from soluble fractions (GPSF, ml/g DM) and gas production from non-soluble fractions (GPNSF, ml/g DM). Nutrient degradability and rumen fermentation parameters, short chain fatty acids (SCFA, mml/ml gas), net energy (NE, MJ/kg DM), metabolizable energy (ME, MJ/kg DM), organic matter digestibility (DOM)%, microbial protein (MP, g/kg DOM), NEL (MJ/Kg DM) , DCPL (g/day), DOMI (g/day) , GED (g/Kg DDM), GED (g/Kg DOM) and TDN (%) are illustrated in Table 7. Figure 2. There were significant ($p<0.05$) variation in terms of the predicted measurements among the tested diets. There were wide range of variations between diet contained 40% from WTM as a replacement of wheat bran protein and control diet in GP, GPNSF and GPSF. Control diet recorded higher ($p<0.01$) GPSF, GPNSF, energy content parameters, MP and DOM in compared with 20% and 40% from WTM as a replacement of wheat bran protein. This might be related to the high cell wall content and tannins of WTM which potentially reducing the *in vitro* fermentation parameters. Plants contain high content of tannins can inhibit feed digestibility and ruminal fermentation parameters through forming complexes with lignocellulose, ether by directly inhibiting rumen microorganisms and the secretion of microbial enzymes (Chung, *et al.* 1998; Nsahlai, Fon and Basha 2011). The increase in DOM of the control diet Table (7) was probably related to its low NDF and ADL contains and high with WTM Table (3).

Table 7. *In vitro* rumen methanogenesis and fermentation parameters of the second experimental concentrate feed mixtures consisting different levels of Waste Trim Mango Trees

Items	Experimental concentrate feed mixtures			SE
	T1	T2	T3	
DDM %	76.80[a]	65.50[b]	56.13[c]	0.544
DOM %	73.76[a]	72.35[a]	68.62[b]	1.033
GP (ml/200mg DM)	48.58[ab]	50.93[a]	43.95[b]	1.737
SCFA (mml/ml gas)	107.39[ab]	112.65[a]	97.14[b]	3.857
GPSF (ML/g DM)	13.919[a]	10.219[b]	9.227[b]	0.542
GPNSF (ml/g DM)	46.19[a]	31.77[b]	27.13[c]	1.175
ME (MJ/Kg DM)	9.44[a]	9.31[a]	9.03[a]	0.311
NEL (MJ/Kg DM)	5.35[a]	5.63[a]	5.06[a]	0.190
NE (MJ/Kg DM)	3.90[a]	3.65[b]	3.56[b]	0.065
DCPL (g/day)	344.80[a]	150.89[b]	97.45b	5.192
DOMI (g/day)	2155.42[a]	1252.32[b]	883.82[b]	1.965
Microbial protein (g/DOM kg)	88.97[a]	87.28[a]	82.78[b]	1.246
GED (g/Kg DMD)	60.76[a]	59.39[a]	55.74[b]	1.009
GED (g/Kg OMD)	64.10[a]	52.80[b]	43.43[c]	0.544
TDN (%)	60.81[a]	60.09[a]	58.58[a]	1.674

a, b, c: within rows, values followed by the same letter are not significantly different (P=0.05). T1 the control diet (concentrate feed mixture). T2 the control diet supplemented with 20% from Waste Trim Mango Trees as a replacement of wheat bran protein. T3 the control diet supplemented with 40% from Waste Trim Mango as a replacement of wheat bran protein.

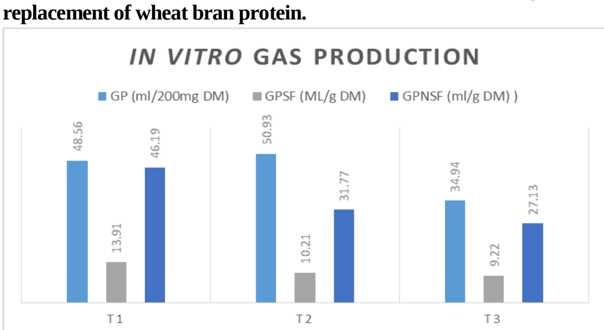

Figure 2. *In vitro* rumen methanogenesis of the first experimental concentrate feed mixtures containing different levels of Waste Trim Mango trees.

Also, ME (MJ/Kg DM), NE, DCPL (g/day), DOMI (g/day), Microbial protein (g/DOM kg), and GED (g/Kg

DDM) were significantly higher with control diet than other experimental diets. No significant differences among all experimental diets were founded on TDN (%). Patra and Saxena (2011) reported that tannin concentration is often considered the most affecting factor on ruminal fermentation parameters. However, Getachew *et al.* (2008) founded it is apparent that the response to tannins could also vary between different tannin sources supplemented at a similar dosage. The obtained results were agreed with Rira *et al.* (2019) who studied the *in vitro* rumen fermentation parameters and methane gas production for Acacia Nilotica, as a plant rich in hydrolyzable tannins. Acacia Nilotica leaves and pods decreased CH4 production ($P < 0.01$). Acacia Nilotica leaves and pods containing tannins decreased rumen fermentation, as indicated by the lower methane gas production and volatile fatty acid productions ($P < 0.01$). Hixson *et al.* (2018) also studied the *in vitro* trails and founded that unsaturated fatty acids and tannin concentration exist in grape marc play a highly role in the anti-methanogenic effect. Rira *et al.* (2015) reported that The *in vitro* trails were used to determine the effect of condensed tannins founded in leaves of Manihot esculenta Gliricidia sepium, and Leucaena leucocephala on methane production and ruminal fermentation characteristics. Methane gas production, rumen VFA concentration, and digestible OM decreased by increased doses of Tannin-rich plants.

CONCLUSION

Deciduous mango leaves and Wasted Trimmings of Mango tannin sources were more effective in suppressing methanogenesis. Results showed that both deciduous mango leaves and Wasted Trimmings of Mango Trees have the potential of reduce it ruminal CH4 production. Therefore, tannins contained in these plants could be of interest in the development of new additives in ruminant nutrition that may be used to reduce Methane emission in the global atmosphere from large scale commercial operation of large and small ruminant animals, which may positively contribute to reducing global warming and untoward climate changes.

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تأثير استخدام أوراق المانجو المتساقطه ونواتج تقليم أشجار المانجو المحتويه على التانين ، على تكوين الميثان ومقاييس التخمي بالكرش.

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قسم الإنتاج الحيواني ، كلية الزراعة ، جامعة بنها ، مصر.

يساهم الميثان من أنظمة الثروة الحيوانية للمجترات في غازات الاحتباس الحراري ، التنينات هي استراتيجية جديده لأضافات الأعلاف التي يمكن أن تخفف من انبعاثات غاز الميثان الناتج من تخمرات الكرش . تميزت هذه الدراسة المعمليه باستجابة تقليل تكوين الميثان في الكرش ومقايس التخمر بالكرش للمنتجات الثانوية لشجر المانجو(أوراق المانجو المتساقطة (DML) و نواتج تقليم أشجار المانجو ((WTM) الغنية بالتانين. أظهرت النتائج المتحصل عليها من التركيب الكيميائي أن WTM سجلت أعلى قيم8.19) CP ٪ (، 4.93) EE٪ (، 52.94) NFE٪ (، 44.23) NDF٪ (، 35.32) ADF٪ (و15.97) ADL . ٪ .(أظهرت نتائج العوامل المضاده للتغذية أن كلا من DML و WTM تحتوي قيم أعلى من التانين (2.0 و 2.4 مجم / 100 جم) على التوالي. و قد لوحظ أيضا أعلى محتوى للغاز GP ، GPSF و GPSNF في DML وسجل 20.44 و 10.04 و 34.49 مل / جم DM على التوالي. تم تسجيل أقل قيمة معنوية للميثان (ML / 200mgDM) باستخدام WTM .أظهرت نتائج الدراسة التجريبية المعملية الأولى وجود اختلافات معنوية(P <0.01) في خصائص التخمير بالكرش عبر مجموعه الكنترول عن الوجبات الغذائية المحتويه علي DML و قد لوحظ أعلى معدل معنوي لـ GP و GPSF و GPSNF في مجموعه الكنترول حيث سجل 49.19 (مل / 200 ملجم) و 14.26 (مل / جم) و 43.45 (مل / جم) على التوالي. كما كانت (ME (MJ / Kg DM و NE و DCPL (جم / يوم) و DOMI (جم / يوم) والبروتين الميكروبي (جم / كجم) و) GED جم / كجم (DMD أعلى بشكل ملحوظ مع مجموعه الكنترول. كانت النتائج التي تم الحصول عليها من التجربة المعملية الثانية أعلى بشكل ملحوظ (p <0.05) في مجموعه الكنترول ف GPSF و GPNSF و SCFAومحتوى الطاقة و OMD و (ME (MJ / Kg DM و NE و (DCPL (g / day و DOMI (جم / يوم) ، بروتين ميكروبي (جم / كجم) ، و) GED جم / كجم (DMD بالمقارنة مع 20٪ و 40٪ من WTM كبديل لبروتين نخالة القمح. وجدت نتائج الدراسة المعمليه لتكوين الميثان أن المنتجات الثانوية لشجر المانجو المحتوية على التانينات لديها القدرة على قمع تكون الميثان. لذلك ، يمكن أن تكون التانينات الموجودة في المنتجات الثانوية لأشجار المانجو ذات أهمية في التطوير كمضاف جديد في تغذية الحيوانات المجترة التي تحد من انبعاثات الميثان في الغلاف الجوي من الحيوانات المجترة في المزارع التجارية الكبيرة.

الكلمات الأساسية: أوراق المانجو المتساقطة ، نواتج تقليم أشجار المانجو ، التانين ، الميثان