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EFFECT OF ADDING DIFFERENT LEVELS OF TANNIN POWDER ON TOTAL GAS AND METHANE PRODUCTION AND *IN VITRO* DIGESTIBILITY

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Abstract

This experiment was conducted to study the effect of adding four different levels of tannin powder (0.2, 0.4, 0.6 and 0.8%) to mixed ration on *in vitro* total gas and methane *in vitro* production, some rumen liquor characteristics and *in vitro* digestibility of dry matter and organic matter after 24, 48, 72 and 96 hr. of incubation periods. The results showed a significant decrease (P <0.01) in total gas and methane production by adding tannin powder at different levels and different incubation periods compared to control treatment. The results showed that nitrogen ammonia concentration decreased significantly (p<0.01) in all treatments after different incubation periods with increased tannin levels. The results showed that the second treatment (0.2% of tannin powder) resulted in a significant increased (p <0.01) *in vitro* dry matter digestibility, organic matter digestibility and metabolizable energy. This was reflected in increased significantly (p<0.01) volatile fatty acids concentration, While the high levels of tannin powder (0.6 and 0.8%) reduced significantly (p<0.01) *in vitro* digestibility of dry matter and organic matter, metabolizable energy and volatile fatty acids concentration, increasing tannin levels significantly (p<0.01) decrease total count of protozoa in the rumen liquor, we conclude that the presence of tannin in ruminant diets at low and medium levels reduce methane production and improve the *in vitro* digestibility.

Key words: Tannin, *In vitro* gas production, *In vitro* digestibility,methane, protozoa.

Introduction

Greenhouse gases are a concern because of their negative effects on global warming and climate change (IPCC, 2007). Methane is a component of greenhouse gases, contributing 9 to 4 percent of total gases (Patra *et al*., 2012), Methane is typically produced from the anaerobic fermentation process of the ruminant digestive system, which produces about 39% of the world's methane (Gerber *et al*., 2013). In addition, 2-15% of the total energy consumed from feed to methane is converted during fermentation in the rumen, thus reducing the efficiency of feed energy use (Kennedy and Charmley, 2012). In recent years researchers have sought solutions to reduce the production of hydrogen gas and methane gas without harming the animal by controlling the ecosystem of the rumen through feed additives such as the addition of vegetable oil (Hassan and Irhim, 2016) or essential oils such as castor oil and flax seed (kutar *et al*., 2017) or are non-food additives such as the addition of nitrates and urea (Hassan and Ali, 2017) or flavonoids of cranberry leaves (Al-Bayati and Hassan, 2018), Some studies have shown that tannin can be added to the diet to improved rumen fermentation with low concentrations (Jayanegara *et al*., 2012). Secondary metabolism product of plant such as tannins also have significant potential to discourage methane production with minimal effect passive of food fermentation in the rumen (Bhatta *et al*, 2013). Tannins are a complex group of phenolic compounds their effect to ruminant may be beneficial or harmful depending on the type of tannin consumed, chemical composition, molecular weight and quantity consumed (Puchala *et al*., 2012). Condensed tannin extracted from chestnut wood (*Castanea sativa*) led to a decrease methane production by 5.5% (Bhatt *et al*., 2009). The production of methane decreases linearly whenever increased amount of tannin in the plants through the effect on rumen microorganisms, especially organisms producing methane. This suggests that tannin is partly responsible for reducing methane production (Huang *et al*, 2010). The aim of this experiment to investigate the effect of adding different levels of tannin to mixed ration in the *in vitro* methane production, which is one method for estimating the nutritional value of feed, in addition to

Table 1: Chemical composition and percentages of ingredients of concentrate (% dry matter).

Metabolizable Energy (MJ/Kg DM)	Nitrogen free extract %	Ash%	Ether Extract %	Crude fiber %	Crude protein %	Organic matter %	Dry matter %	%	Items / Ingredien
12.72	64.89	5.56	4.53	11.00	14.02	84.55	90.12	39	Wheat bran
13.38	77.14	6.43	4.37	3.21	8.85	82.67	89.10	11	yellow corn
11.55	61.28	7.35	1.93	16.57	12.88	80.62	87.97	23	Alfalfa hay
10.11	53.88	6.27	1.32	35.17	3.37	85.10	91.37	17	Barley straw
12.54	42.17	5.01	3.57	5.49	43.77	85.47	90.48	8	Soybean meal
-	-	-	-	-	-	-	-	2	Salt

ME (MJ / kg Dm) = 0.012 × CP + 0.031 × EE + 0.005 × CF + 0.014 × NFE (MAFF, 1975).

estimating *in vitro* digestibility of dry matter and organic matter and some characteristics of rumen liquor.

Materials and Methods

This study was conducted in the college of Agricultural Engineering Sciences, University of Baghdad, in the animal feeding laboratory, department of animal production, period from 14/10/2018 to 28/11/2018. The experiment use mixed ration (40% roughage + 60% concentrate). Table 1, show the components and chemical composition of concentrate.

The tannin powder was obtained from local markets (100% tannin) extracted from oak and shark, added to the mixed ration with four levels (0.2, 0.4, 0.6, 0.8%) to study the effect of tannin on total gas and methane production, some rumen liquid characteristics and *in vitro* dry matter and organic matter digestibility, table 2 shows the chemical composition of mixed ration with different levels of tannin.

In vitro total gas and methane production

In vitro total gas production was estimated by taking 6 replicates per sample according to the Menke and Steingass (1988). 200 mg of experimental mixed ration, adding 20 ml of artificial saliva and 10 ml of rumen liquor extracted from freshly slaughtered lamb. The samples were placed in 100 mL glass syringe, carbon dioxide was added only once time to each syringe, immediately before incubation, the piston was closed to completely remove the air, then incubated in a water bath at 39°C for 24, 48, 72 and 96 hr., with planck for each period of incubation, With moving the syringe twice daily. The syringes was withdrawn to estimate the total gas production and 4 ml of 4% NaOH added to 3 samples only to estimate methane production according to Fievez *et al.,* (2005).

Metabolizable energy (ME), *in vitro* organic matter digestibility (IVOMD), short chain fatty acids (SCFA) and net energy for lactation (NEL) estimate by using total volume of gas production after 24 hr., of incubation period using the following equations :

ME (MJ/kg DM) = 1.06 + 0.157 GV + 0.084 CP + 0.22 CF - 0.081A (Ash)

IVOMD (%) = 14.88 + 0.889 GV + 0.45 CP + 0.651 × A (ASH)

SCFA (m mol /100 ml) = 0.0239 GV - 0.061 according to Menke and Steingass, (1988)

NEL (MJ/Kg DM) = 0.096 × GV + 0.0038 × CP + 0.000173 × EE^2 + 0.54 according to Getachew *et al.,* (1999).

Rumen liquor characteristics

After each incubation periods, estimates the pH valee, NH_3-N, TVFA (Filipek and Dvorak, 2009) and total count of protozoa after 24 hr. of incubation period (Warner, 1962).

Chemical analysis

Experimental diets were determent dry matter, organic matter, crude protein, crude fiber, ether extract and nitrogen free extract according to A.O.A.C., (2005). *In vitro* dry matter and organic matter digestibility estimate according to Tilley and Terry, (1963).

Table 2: Chemical composition and percentages of ingredients of concentrate (% dry matter).

Metabolizable Energy (MJ/Kg DM)	Nitrogen free extract %	Ash %	Ether Extract %	Crude fiber %	Crude protein %	Organic matter %	Dry matter %	Items / Treatment
10.13	41.17	10.51	2.31	26.78	19.25	82.45	92.95	T1Tannin 0%
10.38	44.62	10.35	2.01	24.13	19.25	82.35	92.35	T2 Tannin 0.2%
10.23	42.29	9.53	2.42	26.57	19.21	84.20	93.73	T3 Tannin 0.4%
10.16	42.54	10.33	1.93	25.93	19.30	83.37	93.37	T4 Tannin 0.6%
10.14	43.18	10.54	1.72	25.55	19.07	83.67	94.17	T5 Tannin 0.8%

ME (MJ / kg Dm) = 0.012 × CP + 0.031 × EE + 0.005 × CF + 0.014 × NFE (MAFF, 1975).

Table 5: Effect of addition different levels of tannin powder on metabolizable energy (MJ/ kg DM), *in vitro* organic matter digestibility (%), short chain fatty acids (mmol / 100 ml) and net energy for lactation (MJ/ kg DM) (mean + standard error).

Studied characters / Treatment	Metabolizable energy (MJ/Kg DM)	*In vitro* organic matter digestibility %	Shor chain fatty acids (mmol /100 ml)	Net energy for lactation (MJ/ kg DM)
T1 Control	13.38 ± 0.01a	62.68 ± 0.29a	0.83 ± 0.01a	4.08 ± 0.01a
T2 0.2 %	12.44 ± 0.02b	59.37 ± 0.46b	0.70 ± 0.01b	3.57 ± 0.01b
T3 0.4 %	12.21 ± 0.01c	56.07 ± 0.27c	0.66 ± 0.03c	3.16 ± 0.01c
T4 0.6 %	10.96 ± 0.04d	48.42 ± 0.35d	0.42 ± 0.02d	2.55 ± 0.02d
T5 0.8 %	10.28 ± 0.02e	44.81 ± 0.26e	0.33 ± 0.01e	2.17 ± 0.02e
Moral level	**	**	**	**

T1: control, T2: added 0.2% Tannin powder, T3: added 0.4% tannin powder, T4: added 0.6% tannin powder, T5: added 0.8% tannin powder; ** means that there are significant differences at the probability level (P <0.01).

Statistical Analysis

Data were analyzed statistically using complete randomized design, treatment means were separated using Duncan, (1955), using the SAS, (2012) statistical package, the model as following:

Yij = μ + ti + δij

As:

Yij = the value of viewing studied

μ = general average of the studied recipe

ti = treatment effect i

ijδ = random error which is distributed normal distribution is equal to an average of zero and variance of $e\delta^2$.

Results and Discussion

In vitro total gas and methane production (ml /200 mg Dm)

Table 4, shows decreased significantly (p<0.01) in total gas and methane production with increased tannin levels in the rations during different incubation period compared to control treatment. This was due to the direct effect of tannin on the effectiveness of methane producing microorganisms (Zmora *et al.*, 2012). The results of the current study agreed with reported by Soltan *et al*., (2013), which indicated that the tannin prevents the production of methane both *in vitro* or *in vivo*. It is also consistent with the linear reduction of methane production indicated by Hong *et al*., (2010) due to the addition of 50 mg CT/Kg Dm. It is also consistent with the linear decrease in total gas and methane production due to the addition of tannin Achieved in study of Rira *et al.,* (2015), Contrary to the above, Bhatta *et al.,* (2012) noted that tannin is not effective in reducing methane production due to the addition of low tannin levels, which did not reduce the methane production.

Metabolizable energy, *in vitro* organic matter digestibility, short chain fatty acids and net energy for lactation

The results of the statistical analysis in table 5, show that there were a significantly decreased (p<0.01) in values of the metabolizable energy (from 13.38 to 10.28 MJ / Kg DM), *in vitro* organic matter digestibility (from 62.68 to 44.81%), short chain fatty acids (from 0.83 to 0.33 mmol /100 ml) and net energy for lactation (from 4.08 to 2.17 MJ/ Kg DM) with increase tannin levels. This decline can be explained as a result of estimating the values from total gas production after 24 hr., of incubation periods which decrease as the tannin levels increases and similar results have been achieved from other studies using different types and levels of tannin sources such as the results of Kaplan, (2011) who found a negative correlation between *in vitro* organic matter digestibility and tannin presence in rumen liquor, on the contrary. Theodoridou *et al.*, (2011) explained that intensive tannin did not affect *in vitro* digestibility. The increase tannin also resulted in decrease (p<0.01) short chain fatty acids concentration. This is consistent with Hassanat and Benchaar, (2013), their results confirmed a linear decrease *in vitro* short chain fatty acids when the tannin levels increased from 20 to 200 mg / Kg DM.

Table 6: Effect of addition tannin powder on *in vitro* dry matter and organic matter digestibility (%) and metabolizable energy (MJ /kg DM) (mean + standard error).

Studied characters / Treatment	Metabolizable energy (MJ/Kg DM)	*In vitro* organic matter digestibility %	*In vitro* dry matter digestibility %
T1 Control	10.76 ± 0.01b	70.61 ± 0.54 c	68.19 ± 0.14 c
T2 0.2 %	11.82 ± 0.13a	78.95 ± 0.05 a	77.54 ± 0.46a
T3 0.4 %	10.96 ± 0.05b	72.06 ±0.32 b	70.03 ± 0.04b
T4 0.6 %	8.52 ± 0.02c	56.10 ± 0.21 d	55.04 ± 0.16 d
T5 0.8 %	7.88 ± 0.09d	51.89 ± 0.09e	50.09 ± 0.24 e
Moral level	**	**	**

T1: control, T2: added 0.2% Tannin powder, T3: added 0.4% tannin powder, T4: added 0.6% tannin powder, T5: added 0.8% tannin powder; ** means that there are significant differences at the probability level (P <0.01).

Table 7: Effect of adding different levels of tannin powder on concentration of rumen ammonia nitrogen (mg /100 ml) after different incubation periods (mean + standard error).

Treatment	96 hr.	72 hr.	48 hr.	24 hr.
T1 Control	26.53 ± 0.34 a	30.13 ± 0.30 a	32.53 ± 0.24 a	34.79 ± 0.23a
T2 0.2 %	19.20 ± 0.18 b	20.95 ± 0.22 b	24.63 ± 0.30 b	28.19 ± 0.18 b
T3 0.4 %	16.85 ± 0.30 c	17.84 ± 0.15 c	20.92 ± 0.21 c	24.17 ± 0.28 c
T4 0.6 %	11.18 ± 0.21 d	13.09 ± 0.25 d	16.04 ± 0.16 d	18.85 ± 0.17 d
T5 0.8 %	10.38 ± 0.16 e	10.74 ± 0.22 e	11.95 ± 0.19 e	14.87 ± 0.12 e
Moral level	**	**	**	**

T1: control, T2: added 0.2% Tannin powder, T3: added 0.4% tannin powder, T4: added 0.6% tannin powder, T5: added 0.8% tannin powder; ** means that there are significant differences at the probability level (P <0.01).

In vitro dry matter and organic matter digestibility (%) and metabolizable energy (MJ/ kg DM)

The results of table 6, indicate a significant increased (P<0.01) *in vitro* dry matter and organic matter digestibility and metabolizable energy in the second treatment (0.2% tannin powder) compared to the control treatment (without tannin) (77.54, 78.95% and 11.82 MJ/Kg DM respectively) and the lowest value was recorded for *in vitro* dry matter and organic matter digestibility and metabolizable energy in the fifth treatment (0.8% tannin powder) (50.09, 51.89% and 7.88 MJ/Kg DM respectively). This improvement (0.2% tannin powder) can be explained as the best among the treatments may be as a result of low protein degradation by microorganisms in the first phase of *in vitro* digestibility leading to exposure to enzymatic digestion in the second phase of *in vitro* digestion. Thus increasing the utilization of amino acids and this corresponds to the amount of increase achieved in the study of Widiawati *et al.,* (2013) when the addition of different levels of tannin which led to high *in vitro* organic matter digestibility, Plaizier *et al*., (2000) also noted that tannin increases *in vitro* digestibility when added by 3.0-5.0%, However, the rate of improvement *in vitro* digestibility and energy was reduced by increasing levels of tannin powder because high levels of tannin are complex with both protein and carbohydrates, which reduces the digestion of nutrients.

Characteristics of rumen liquid

• ammonia Nitrogen concentration (NH_3-N):

The data in table 7, show that the ammonia nitrogen concentration decreased significantly (p<0.01) in all treatments and during different incubation periods with increased tannin level, the value after 24, 48, 72 and 96 hr. were from 34.79 to 14.87, from 32.53 to 11.95, from 30.13 to 10.74 and from 26.53 to 10.38 mg / 100 ml respectively. This decrease may indicates low protein degradation in the rumen because of the complex of tannin with protein. This applies on effectiveness of tannin in improving the utilization of nitrogen (Avila *et al*., 2016 and Orlandi *et al*., 2015), Contributes to the reduction of urea nitrogen and increased nitrogen retention in the body (Karen and Karen, 2018). This is consistent with both Aufrere *et al.,* (2013) and Soltan *et al.,* (2013).

Volatile fatty acids Concentration (mmol /100 ml)

Table 8, shows the effect of adding different levels of tannin powder on short chain fatty acids concentration after different incubation period. There were no significant differences in volatile fatty acids concentration after 24 hr. of *in vitro* incubation in the first, second and third treatment. This is consistent with Silanikove *et al.,* (2006) and Getachew *et al.,* (2008). Where found that low levels of intensive tannin did not affect the concentration of short chain fatty acids on the contrary, T4 and T5 decreased significantly (p<0.01) compared with others treatment, this decline agree with Martin *et al.,* (2010) who found a decrease in short chain fatty acids concentration with increased tannin level because of the complexity that prevent the breakdown of carbohydrates in the rumen by microorganisms lead to the availability of short chain fatty acids to longer hours to increase their use in manufacturing microbial protein (Beauehemin *et al*., 2014; Silanikove *et al*., 2006).

Table 8: Effect of adding different levels of tannin powder on concentration of volatile fatty acids (mmol/100 ml) after different incubation periods (mean + standard error).

Treatment	96 hr.	72 hr.	48 hr.	24 hr.
T1 Control	59.83 ± 0.11 b	63.02 ± 0.29 c	70.94 ± 0.10 b	80.87 ± 0.08a
T2 0.2 %	60.54 ± 0.18 a	64.84 ± 0.26 b	72.77 ± 0.47 a	81.26 ± 0.58 a
T3 0.4 %	61.54 ± 0.64 a	65.86 ± 0.18 a	72.16 ± 0.19 a	81.08 ± 0.16 a
T4 0.6 %	59.66 ± 0.69 b	62.29 ± 0.21 c	70.87 ± 0.15 b	75.84 ± 0.12 b
T5 0.8 %	55.29 ± 0.16 c	60.77 ± 0.22 d	64.19 ± 0.20 c	71.01 ± 0.49 c
Moral level	**	**	**	**

T1: control, T2: added 0.2% Tannin powder, T3: added 0.4% tannin powder, T4: added 0.6% tannin powder, T5: added 0.8% tannin powder; ** means that there are significant differences at the probability level (P <0.01).

pH

The results of the statistical analysis of pH data in the present study showed that the effect of adding different levels of tannin powder on the pH value of the rumen fluid had the highest value (p<0.01) after 24 hr., of *in vitro* incubation were 7.0 in the third treatment (0.4% tannin powder) and the

Table 9: Effect of adding levels of tannin powder on pH and protozoa (cell ×10^5/ ml) after different incubation periods (mean + standard error).

Studied Characters	Protozoa (cells × 10^5 / ml)	pH			
		Incubation period			
Treatment		96 hr.	72 hr.	48 hr.	24 hr.
T1 Control	3.83 ± 0.02a	7.53 ± 0.06 a	7.27 ± 0.07 a	6.93 ± 0.06 a	6.53 ± 0.03c
T2 0.2 %	3.64 ± 0.01 b	6.77 ± 0.03 b	6.87 ± 0.03 c	6.87 ± 0.03 ab	6.93 ± 0.06 a
T3 0.4 %	3.45 ± 0.01c	6.87 ± 0.03 b	7.07 ± 0.03 b	6.87 ± 0.03 ab	7.03 ± 0.03 a
T4 0.6 %	2.86 ± 0.01 d	6.63 ± 0.03 c	6.63 ± 0.03 d	6.73 ± 0.03 bc	6.77 ± 0.03 b
T5 0.8 %	2.06 ± 0.01 e	6.87 ± 0.03 b	6.63 ± 0.03 d	6.67 ± 0.03 c	6.73 ± 0.03 b
Moral level	**	**	**	**	**

T1: control, T2: added 0.2% Tannin powder, T3: added 0.4% tannin powder, T4: added 0.6% tannin powder, T5: added 0.8% tannin powder; ** means that there are significant differences at the probability level (P <0.01).

lowest value in the control treatment without the addition of tannin powder amounted to 6.5, also table 9, shows that the pH value after 48, 72 and 96 hr. of *in vitro* incubation decreased in all treatments compared to the control treatment free of additive (6.9, 7.3 and 7.5, respectively) and the lowest pH value was recorded in the fifth treatment (0.8% tannin powder) 6.7, 6.6 and 6.9, respectively. As shown in the data in table 9, the addition of different levels of tannin powder after different *in vitro* incubation hr., it did not have a significant impact on the fluctuation in the pH value which ranged between 7.5-6.5 which are suitable for the activity of microorganisms that analyze the fibers and protein in the rumen (Hungate, 1966); Carrasco *et al*., (2017) recorded pH values approximate the values shown by the current study results where it recorded 6.5-7.0 when adding 0.4% Tannin, while Zawadzki *et al*., (2010) note the low value pH when using 3 and 4 g tannin condensed / kg dry matter.

Protozoa

Table 9, shows that addition of tannin powder to the mixed ration resulted in a significantly decreased (P<0.01) in protozoa number with increased tannin level after 24 hr. of *in vitro* incubation (from 3.83 to 2.06 cells×10^5/ ml). This is consistent with the results of both Animut *et al*., (2013); Sallam *et al*., (2010); Longo *et al*., (2013). Where the researchers pointed to a linear decline in the protozoa population when adding tannin in different levels, May be one of the reasons for the decline protozoa population after 24 hr. of incubation period the effect of tannin on the unavailability of iron and calcium for protozoa utilizing, which effect on inability to multiply and growth (Ri carde-da Silva *et al*., 1991).

Conclusion

We concluded that low levels of tannin (0.2%) in the diet improved the *in vitro* dry and organic matter digestibility due to conservation of feed energy with decreased methane production as a result of organic fermentation processes by inhibiting the growth of methauogenesis organisms.

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