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IN- SITU DEGRADABILITY AND IN VITRO GAS PRODUCTION OF RHODES (*CHLORIS GAYAN*) AND BUTTERFLY (*CLITORIA TERNATEA*) AS AFFECTED BY STAGE OF GROWTH, PHOSPHOROUS FERTILIZATION AND INTERCROPPING

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ABSTRACT

Nutritive value and fermentation characteristics of *Chloris gayana* as sole crop, *Clitoria ternatea* as sole crop and a mixture of both *Chloris gayana* and *Clitoria ternatea* with the effect of stage of growth and phosphorous fertilization was determined using two different techniques: (i) the in vitro gas production and (ii) the in situ nylon bag degradability technique. Samples of 45 and 60 days from sowing were used. The phosphorous fertilization was applied at a rate of 0, 50, 75 and 100 kg P₂O₅ / ha. Samples were incubated in situ and in vitro for 4, 8, 16, 24, 48, 72 and 96 h. In situ and in vitro DM degradation kinetics were described using the equation $y = a + b(1 - e^{-cy})$. For all forages, readily degradable fraction (a) and effective degradability at all out flow rates were higher than the late stage of growth. However, slowly degradable fraction (b) and degradation rate (c) were higher in the late stage of growth. The potential degradability (PD) is similar in both time of cutting. Phosphorous fertilization had no significant effect on in situ parameters. The gas produced was found to be higher in the early stage of growth than late stage of growth. For gas production parameters, late stage of growth for Rhodes grass in the pure stand and in the mixture had higher value than the early stage of growth. But for clitoria early stage of growth had a higher value than late stage of growth. Also, Phosphorous fertilization had no significant effect on in situ parameters.

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INTRODUCTION

In Sudan, as in various parts of tropical areas, most small scale farmers rely on grasses as material feed input for animal maintenance. However, forage quality such as (digestibility, protein content and mineral content) decline during dry seasons which leads to nutritional imbalance that causes weight loss (Balgees et al., 2013). The rate and extent of fermentation of dry matter (DM) in the rumen are very important determinants for the nutrients absorbed by ruminants (Kamalak et al., 2004). There is a wide array of methods available to assess the digestibility of ruminant forages. These methods consider the differences between the apparent digestibility (overall digestive tract), the ruminal digestibility (degradability) and the stomach digestibility (actual, bypass, digestibility) (Margareta et al., 2005). The techniques used to

assess the nutritive value can be classified as follows: a. methods for in vivo digestibility; b. methods for in vitro digestibility – (in situ incubation with ruminal fluid or with cellulolytic enzymes); c. methods derived from the in sacco method d. assessment using the regression equations; e. assessment using markers; f. assessment using NIRS. Irrespective of the used techniques, compared to the in vivo technique, the analytical advantages are increasingly visible by simplification and standardization, followed by the use for forage assessment and for the development of rumen models. Starting from the advantages of the in vitro, cellulase method for digestibility assessment (no need to use animals with fistulae, shorter working time, accuracy etc.) (Margareta et al., 2005) the aim of the current study is to compare between insitu DM deteterminen the nutritive value of Rhodes grass(*Cholris gayag*) and butterfly bean(*Clitoria ternatea*) and their response to harvesting time, phosphorous fertilization and intercropping, using in situ degradability and in vitro gas production test.

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MATERIALS AND METHODS

The study was carried out at the University of Khartoum Demonstration Farm. The locality is the arid zone with hot summer and short rainy season from July to September. Samples of the forages were collected from the field at 45 days from sowing.

Chemical analysis

The samples were to analysis for proximate component. chemical analysis. DM, Ash and CP were determined according to AOAC methods (AOAC, 1990). NDF, ADF and ADL were determined according to Georing and Van Soest (1970).

In vitro gas production test

The in vitro gas production of Rhodes grass and clitoria ternate as pure stand and mixture was determined according to the procedure of Menke and steingass (1988). Rumen fluid was collected from two rumen fistulated local breed calves at the morning before feeding. The rumen fluid was then transferred through two layers of cheese cloth into a warm flask. A sample of about 200 mg DM weight into 100 ml capacity glass syringe calibrated for gas measurement in duplicate. The syringes were incubated in a water bath at 39 °C and covered with a Perspex lid fitted with holes to hold the syringes upright in the water bath. The gas volume produced was recorded at 3, 6, 12, 24, 48, 72 and 96 hours. The gas produced was determined in three runs for each sample. In every run, blanks (buffer rumen fluid solution without samples) were included. The gas volume measured was corrected for the blanks. The potential and rate of gas production was fitted to the equation $Y = b(1 - e^{-ct})$, where Y is the volume of gas production at time t, b the potential gas production and c the rate of gas production (Orskov and McDonal, 1979). The metabolizable energy (ME) was estimated from the gas data using the equation $ME = 1.242 + 0.146GP + 0.007CP + 0.0224EE$, where GP is the net gas production (ml/200mg DM) in 24 h incubation Menke and steingass (1988), CP and EE is the crude protein and ether extract respectively.

In situ degradability

In situ degradability of the samples was determined according to procedure described by (Orskov and McDonal, 1979). Each forage sample (Rhodes grass and clitoria ternate as pure stand and mixture) weighing 5 g was transferred into nylon bags with a dimension of 8 x 14 cm and a porosity of 40-45mm and incubated in duplicate in two rumen fistulated steers. The samples containing nylon bags were incubated for 3, 6, 12, 24, 48, 72 and 96 hours, and there after hand washed using tap water. Zero hour solubility was also determined by hand washing samples containing in nylon bags in a similar way to the incubated feed samples. The washed samples were air dried in oven at 105 °C to constant weight to determine dry matter degradability. The in situ degradability parameters were fitted using the equation $P = a + b(1 - e^{-ct})$, where Y is the DM disappearance at time t, a immediately soluble (wash loss), b the slowly degradable fraction and c the rate of degradation (Orskov and McDonal, 1979).

RESULTS AND DISCUSSION

Table (1) summarized the Effect of intercropping on the chemical composition of Clitoria ternate, Chloris gayana when

harvested after 45 days from sowing. As general butter fly bean as pure stand and in the mixture had a higher ($P < 0.05$) OM, CP and EE and lower NDF and ADF than Rhodes grass in both pure stand and in intercropping system. Clitoria as pure had a higher value ($P < 0.05$) CP, EE, but ADF was found to be insignificant ADF was ADF, while had lower and NDF the clitoria in the mixture but with no significant difference. Rhodes grass in association with legume had high but significant OM and CP, and significantly higher EE and significantly ($P < 0.05$) lower NDF and ADF. As expected, butter fly bean had a higher CP content ranging from 206- 216 g/kg than Rhodes grass which had CP from 103.2- 115.4 g/kg. (Sleugh *et al.*, 2000; Zemenchik, 2002) reported that, Legumes have higher nutritive value than grass species so growing mixtures of grasses and legumes can improve forage quality compared to grass monocultures. The typical and obvious advantages of intercropping was observed for rhodes grass. The CP, OM and EE were higher in rhodes grass when growing in intercropping system than the monoculture, in addition, NDF and ADF of rhodes grass in monoculture outnumbered these value for the grass in the mixture. The results in this study were in agreement with many researchers (Bakhashwain, 2010 and Ibrahim *et al.*, 2006), who reported that, introducing legume fodder plants with cereal plants or grasses, will increase protein contents. Generally, mixing of legumes in cereal or grass fodder is a better way to increase the quality of cereal or grass fodder. That is because fodder quality of grassy hay is lower than that required to meet production goals for many livestock classes, whereas legume-cereal mixtures are important protein and carbohydrate sources for livestock (Karadau, 2003). The higher CP of Rhodes grass when associated with Clitoria ternate is higher may be attributed to the accumulation of the N fixed by the legumes. The symbiotic N fixed by the legumes and its effect on the grasses has been reported by Shehu (1999) who reported that the crude protein content of the sorghum stem was higher in mix crop than in sole crop,

In situ dry matter degradability

Table 3 shows the effect of different times of cutting on in situ degradation characteristics for different types of forages. The data revealed that at early stage of growth, readily degradable fraction (a) and effective degradability at all out flow rates were higher than the late stage of growth. However, slowly degradable fraction (b) and degradation rate (c) were found to be higher in the late stage of growth. The potential degradability (PD) is similar in both time of cutting. The new protein systems (AFRC 1993) base the evaluation of dietary protein on the concept of readily degradable fraction and slowly degradable fraction for protein to meet the needs of rumen microflora, and undegradable dietary protein to meet host animal requirements. The readily degradable fraction comprises non-protein-nitrogen, free amino acids and small protein molecules (AFRC 1993). The difference in dry matter degradability between the early and the late stage of maturity could be due to the fact that in mature Sporobolus there is greater deposition of lignin and other indigestible secondary cell wall constituents. In addition, Marandure and Masama (2012) reported that the increase of DM degradability in early stage of growth may be due to the fact that during the juvenile stage of growth there is rapid accumulation of dry matter as most energy and protein are partitioned towards the vigorous growth that occurs at this stage.

Table 1. Effect of intercropping on the chemical composition of Clitoria ternate, Chloris gayana when harvested after 45 days from sowing

Forage type	OM (g/kg)	CP (g/kg)	EE (g/kg)	NDF (g/kg)	ADF (g/kg)
Rhodes grass pure stand	895.9c	103.2de	5.9e	749.2b	493.8cd
Rhodes grass in the mixture	905.5bc	115.4cd	22.3ab	777.2a	469.7e
Clitoria ternate pure stand	911.7ab	216.0a	25.7a	617.3d	402.2e
Clitoria ternate in the mixture	915.0a	206.3bc	18.6bcd	624.5d	338.2e
SEM	0.369	0.5812	01705	0.708	0.8018

OM = organic matter; CP = crude protein; EE = ether extract; NDF = neutral detergent fibre; ADF = acid detergent fibre.
Means with different subscription in the same column differ significant.

Table 2. Rmen degradability kinetics (%) for Rhodes grass as pure stand (R), Clitoria in the pure stand (C), Rhodes grass in the mixture (MR), Clitoria in the mixture (MC) as affected by different time of cutting

		a	B	c	PD	ED 0.02	ED 0.05	ED 0.08
R	45	17.77cd	56.90ab	0.028cd	74.67ab	47.05c	35.76c	30.90c
	60	19.15cd	53.78bc	0.030cd	72.92cd	54.68c	37.50c	32.55c
MR	45	20.32c	49.13cd	0.025cd	69.91cd	47.41c	36.65c	32.05c
	60	15.67d	60.66a	0.018cd	76.31cd	43.05d	31.05d	26.40d
C	45	32.53a	37.47e	0.050b	70.02bc	59.07a	54.15a	46.92a
	60	30.31a	34.07e	0.057ab	64.35ab	60.23b	47.90c	44.18ab
MC	45	31.82a	38.27e	0.035c	70.07c	55.58b	47.28b	43.32b
	60	25.31b	46.51e	0.069d	71.80d	48.78a	51.10a	45.86ab
	SEM	1.37	2.15	0.0041	1.7	0.838	0.96	1.02

R = Rhodes grass; c = Clitoria; MR = Rhodes in mixture; MC = Clitoria in mixture.

Means with different subscription in the same column differ significant.

a = readily degradable fraction, b = slowly degradable fraction, c = degradation rate, PD = potential degradability, ED = effective degradability.

Table 3. Rumen degradability kinetics (%) for Rhodes grass as pure stand (R), Clitoria as pure stand (C), Rhodes grass in the mixture (MR), Clitoria in the mixture (MC) as affected by different level of phosphorous fertilization

		a	B	c	PD	ED 0.02	ED 0.05	ED 0.08
R	0P	16.9ef	56.8bc	0.03def	73.7bc	42.2ef	34.9e	30.1e
	1P	22.2de	46.1de	0.04cde	68.3cde	53.4d	43.4d	38.2d
	2P	18.0ef	36.4a	0.02fg	84.8a	48.4e	35.8e	30.7e
	3P	16.8ef	42.0bcd	0.02fg	68.9bcd	43.6g	32.5e	27.9e
MR	0P	20.0e	55.8bc	0.02g	75.8b	43.6g	32.8e	28.8e
	1P	20.7bcd	54.9bc	0.02g	75.6b	44.6fg	33.9e	29.8e
	2P	14.3f	59.6ab	0.03fg	70.8bcd	47.3ef	34.1e	28.5e
	3P	16.9ef	49.4cde	0.03efg	66.3bcd	45.6efg	34.7abc	29.9e
C	0P	26.7cd	42.6ef	0.07ab	69.4bcd	58.8ab	50.4ab	45.6abc
	1P	30.8abc	35.7gh	0.06abc	66.6e	56.8bcd	49.5abc	45.5abc
	2P	35.3a	32.3h	0.04cde	67.6cde	57.3abc	34.1ab	28.5ab
	3P	32.8ab	32.2h	0.05cde	65.3e	54.6cd	47.9e	44.5bc
MC	0P	29.7bc	40.8ef	0.05cd	69.5bcd	57.1abc	48.3bc	43.6bc
	1P	27.2bcd	45.8de	0.04def	73.0bcd	55.8bcd	46.2cd	41.6cd
	2P	31.5abc	39.1fg	0.07a	70.6bcd	60.2a	53.0a	48.9a
	3P	26.9cd	43.8de	0.05bc	70.7bcd	58.6bcd	49.4abc	44.4bc
	SEM	1.94	3.05	0.0058	2.41	1.19	1.36	1.44

R Rhodes grass; c = Clitoria; MR = Rhodes in mixture;

MC = Clitoria in mixture.

0P, 1P, 2P and 3P are 0, 50, 75 and 100 kg/ha the rate of phosphorous application

a = readily degradable fraction, b = slowly degradable fraction,

c = degradation rate, PD = potential degradability, ED = effective degradability.

Table 5. Rumen degradation of DM (%) for rhodes grass, clitoria ternatea and the mixture as affected by time of cutting

		4	8	16	24	48	72	96
R	45	24.45	28.02	31.94	43.61	54.13	60.46	64.20
	60	25.26	31.03	36.77	37.38	57.28	66.45	72.00
MR	45	28.09	30.29	30.34	40.48	59.86	61.14	62.75
	60	21.64	22.27	24.25	31.72	56.14	56.95	62.31
C	45	40.76	54.81	52.72	61.07	63.18	69.09	70.33
	60	38.89	48.20	57.79	58.35	60.50	61.25	62.60
MC	45	36.97	42.60	47.06	49.92	66.14	65.42	66.43
	60	38.11	49.25	57.25	59.30	62.00	62.44	70.30

R Rhodes grass; c = Clitoria; MR = Rhodes in mixture; MC = Clitoria in mixture.

Table 6. Rumen degradation of DM (%) for rhodes grass, clitoria ternatea and the mixture as affected by phosphorous fertilization

		4	8	16	24	48	72	96
R	0P	24.04	25.93	31.88	46.17	50.17	61.81	62.67
	1P	30.25	30.64	35.14	59.90	58.87	65.63	69.23
	2P	25.34	29.69	31.57	38.51	56.80	62.20	66.60
	3P	21.78	22.75	29.62	37.14	50.34	57.90	57.94
MR	0P	25.27	26.81	28.66	43.71	48.90	56.03	57.40
	1P	28.03	28.95	27.79	35.88	51.44	59.77	58.95
	2P	24.20	25.00	30.30	38.33	62.46	65.29	66.37
	3P	24.69	25.15	41.75	41.07	53.41	61.78	62.07
C	0P	38.38	38.89	51.06	63.48	65.64	67.80	68.51
	1P	39.15	40.39	51.40	59.38	58.95	67.46	69.44
	2P	42.42	41.75	50.77	58.47	60.47	65.30	70.06
	3P	38.57	40.49	48.58	55.54	57.37	61.98	64.47
MC	0P	36.79	40.67	47.05	56.08	63.56	64.76	72.74
	1P	31.04	38.36	45.41	50.14	63.95	66.52	70.56
	2P	43.62	50.76	56.68	58.68	67.53	67.66	71.50
	3P	31.71	41.50	48.81	52.81	69.87	67.77	71.03

R Rhodes grass; c=Clitoria; MR=Rhodes in mixture; MC = Clitoria in mixture. 0P, 1P, 2P and 3P are 0, 50, 75 and 100 kg/ ha the rate of phosphorous application

Table 7. Gas production constant (ml/200 mg DM) at the different hours of incubation of Rhodes grass as pure stand (R), Clitoria in the pure stand (C), Rhodes grass in the mixture (MR), Clitoria in the mixture (MC) as affected by different time of cutting

	R		MR		C		MC		SEM
	45	60	45	60	45	60	45	60	
a	-1.62b	-1.44b	-1.7ab	-1.95b	1.2a	-2.04b	-1.7b	-2.68b	0.88
B	42.0ab	65.9a	40.4ab	50.1ab	29.0b	26.7b	36.5b	33.0b	8.95
C	0.05b	0.030b	0.05b	0.04b	0.09a	0.09a	0.09a	0.08a	0.01
a+ b	40.39ab	64.45a	38.69b	48.07ab	30.161b	24.635b	35.4b	30.271b	8.78

R = Rhodes grass; c=Clitoria; MR=Rhodes in mixture; MC = Clitoria in mixture. a= readily degradable fraction, b= slowly degradable fraction, c= degradation rate, (a+b) = potential degradability , Means with different subscription in the same rows differ significant.

Table 8. Gas production volume (ml/200mgDM) for Rhodes grass as pure stand (R), Clitoria as the pure stand (C), Rhodes grass in the mixture (MR), Clitoria in the mixture (MC)

	R		MR		C		MC	
	45	60	45	60	45	60	45	60
3	5.25	2.5	5.22	4.00	9.10	7.56	9.47	7.13
6	11.39	8.45	11.76	11.37	21.55	24.74	23.13	23.62
12	22.01	16	21.93	19.61	34.06	38.87	35.77	36.11
24	40.54	35.67	41.37	41.21	42.62	50.34	42.87	46.21
48	54.14	51.58	56.53	60.56	49.84	59.00	49.66	55.19
72	58.18	58.58	60.57	65.85	51.96	60.98	52.65	57.23

R Rhodes grass; c=Clitoria; MR=Rhodes in mixture; MC = Clitoria in mixture.

Table 9. Gas production constants (ml/200 mg DM) at the different hours of incubation, for Rhodes grass and Clitoria ternate and the mixture at two time of cutting

		a	b	c	a+b
45	R	-1.62b	41.97ab	0.05b	40.39ab
	MR	-1.7ab	40.41ab	0.0488b	38.69b
	C	1.2a	28.96b	0.0925a	30.161b
	MC	-1.72b	36.53b	0.085a	35.4b
60	R	-1.44b	65.89a	0.030b	64.45a
	MR	-1.95b	50.03ab	0.0388b	48.07ab
	C	-2.04b	26.67b	0.0863a	24.635b
	MC	-2.68b	32.96b	0.0788a	30.271b
SEM		0.88	8.95	0.01	8.78

R Rhodes grass; c=Clitoria; MR=Rhodes in mixture; MC = Clitoria in mixture, a= readily degradable fraction, b= slowly degradable fraction, c= degradation rate.

Means with different subscription in the same rows differ significant.

Table 10. Gas production constants (ml/200 mg DM) at the different hours of incubation, for Rhodes grass and Clitoria ternate and the mixture at different level of phosphorous fertilization

		A	b	c	a+ b
R	0P	-2.5b	49.67ab	0.045d	47.17ab
	1P	-1.02ab	39.42b	0.035d	38.4b
	2P	-0.86ab	44.50b	0.045d	43.64b
	3P	-0.176ab	82.13a	0.040d	80.37a
MR	0P	-3.46b	48.27ab	0.04d	44.18ab
	1P	-0.61ab	44.36b	0.050cd	43.75b
	2P	-3.12b	32.76b	0.050cd	32.23b
	3P	-0.173ab	52.90ab	0.102d	52.72ab
C	0P	1.22a	29.21b	0.088abc	56.54b
	1P	-0.08ab	28.36b	0.070bcd	28.27b
	2P	-0.30ab	25.12b	0.087abc	24.81b
	3P	-2.50b	28.57b	0.112a	26.06b
MC	0P	-2.62b	36.91b	0.068bcd	34.29b
	1P	-2.34ab	38.98b	0.052cd	36.63b
	2P	-2.13ab	32.76b	0.105ab	30.62b
	3P	-0.52ab	30.32b	0.035ab	29.80b
SEM		1.258	12.661	0.0138	12.42

R Rhodes grass; c=Clitoria; MR=Rhodes in mixture; MC = Clitoria in mixture, a= readily degradable fraction, b= slowly degradable fraction, c= degradation rate.

Means with different subscription in the same rows differ significant.

Table 11. Gas production volume (ml/200mgDM) for Rhodes grass as pure stand (R), Clitoria as the pure stand (C), Rhodes grass in the mixture (MR), Clitoria in the mixture (MC) as affected by phosphorous fertilization

		3	6	12	24	48	72
R	0P	3	9.62	17.17	36.09	54.50	60
	1P	2.75	8.62	16.92	40.00	55.50	62.42
	2P	2.5	8.45	16.07	35.47	51.58	58.28
	3P	1.75	7.12	13.92	30.34	44.75	52.42
MR	0P	2.25	8.37	15.42	37.59	56.25	58.42
	1P	4.25	11.87	21.17	44.34	64.75	72.17
	2P	4.25	12.12	20.92	42.34	63.00	62.42
	3P	5.25	13.12	21.17	40.09	58.25	65.42
C	0P	7.5	25.87	40.67	50.84	58.50	61.17
	1P	6	21.37	34.97	49.59	60.75	64.67
	2P	8.5	29.62	43.67	53.84	61.25	63.67
	3P	8.25	22.12	36.17	47.09	55.50	58.42
3P	0P	5.5	13.62	21.17	28.59	35.75	38.17
	1P	9.25	30.12	44.42	55.84	64.25	62.17
	2P	5.75	20.62	37.42	48.09	59	63.42
	3P	8.00	30.12	41.42	52.34	61.75	65.17

R Rhodes grass; c=Clitoria; MR=Rhodes in mixture; MC = Clitoria in mixture.

a= readily degradable fraction, b= slowly degradable fraction, c= degradation rate.

Means with different subscription in the same rows differ significant.

The general decrease in CP with maturity is supported by Alemayehu (2006) who indicated that range forage species vary in quality and quantity from time to time and from place to place. It is during the growth stages that plants are most nutritious (Alemayehu, 2006). Similarly the increases in EE, ash, ADF and NDF with maturity also follows the trends that were recorded by Theander and Aman (1984) who indicated that as plants mature, CP, P and the more readily digested carbohydrates decrease, in contrast, fiber, lignin and cellulose increase. Once mature (Alemayehu, 2006). Jocelyne *et al.* (2003) reported that The degradability of dry matter decreased progressively with the maturity of the plant ($p > 0.05$). The reduction in readily degradable fraction content of the legumes with increasing maturity is due to a decline in the soluble cell contents while cell wall contents increased (Balde *et al.*, 1993). Concerning the effect of different types of forages on DM degradability (table 3), readily degradable fraction (a) was found to be higher in pure clitoria (C) ($P < 0.05$) followed by clitoria in a mixture (MC) which in turn found to be higher than Rhodes grass (R) in a pure stand and the least mean value in Rhodes grass in the mixture (MR). The slowly degradable fraction (b) was found to be higher in (R) and (MR) ($P < 0.05$) than (MC) and (C). The degradation rate (c) was found to be

similar for (C) and (MC) and greater ($P < 0.05$) than (R) and (MR). The potential degradability (PD) is similar for forages (R), (MR). ranked as follows: (MC), (C), (R) and (MR). It could be concluded that, the high readily degradable fraction content of the legumes even, at advanced stages of maturity, shows that they can supply sufficient quantities of N to meet rumen microbial requirements (Mupangwa *et al.*, 2003). The high readily degradable fraction observed in this study for Clitoria pure stand and in the mixture than both rhodes grass probably due the contents of different proportion of digestible fiber particles which led to difference in degradation and subsequently potential degradation in the rumen, Therefore, feeding of Clitoria to the ruminants may provide adequate nutrients and may reach to potential intake and digestion. More over (Margareta *et al.*, 2005) stated that the digestibility of Alfalfa was found to be higher than barely silage of Sudan grass silage. The effect of phosphorous fertilization on Dry matter degradability was illustrated in Table 7. The results showed that phosphorous fertilization affects the potential degradability (PD) and the effective degradability (ED) at all out flow rates significantly ($P < 0.05$). Level 2 of phosphorous fertilization recorded the highest mean value followed by level 1 and zero level for (PD) and (ED). Readily degradable

fraction (a) slowly degradable fraction (b) and degradation rate did not affect significantly by phosphorous fertilization, however, the ranking phosphorous fertilization was as follows $2 > 1 > 0 > 3$. These results were in conformity of (Tolera et al., 2008) who worked on fertilizer application on nutritive value of durum wheat Straw, found that phosphorous fertilization had no significant effect on dry matter degradability.

Gas production test

The volume of gas produced by different types of forage is presented in table (8) the gas produced was found to be higher in the early stage of growth than late stage of growth. Low gas production in late stage due to increase in insoluble fractions over the soluble fractions as reported, which described negative effect on digestion of the cell wall associated compound, like phenolic acids and the cross-linkage of lignin and wall polysaccharides. As the fermentation process is partially regulated by the fibrous content of the feeds, the relatively low content of fibre can facilitate the colonisation of the feed by the microbial rumen population, which in turn might induce higher fermentation rates, therefore improving digestibility (Akinfemi et al., 2012). These results similar to that obtain by (Bayatkouhsar, 2012) for ensiled corn forage. Also similar result was found by (Van Soest, 1994) who stated that, the legume cell wall contains about twice the lignin as grass but ferments faster than grass at the same stage of maturity. This could be attributed to availability of immediately fermentable carbohydrates in the young forages and legumes (soluble fractions). Contradicting results were obtained (Kazemi et al., 2009) who noted that, the cumulative gas production for the Sorghum halepense was significantly high in comparison to other weed forages. Sallam et al. (2008) reported that cell wall content (NDF and ADF) were negatively correlated with gas production at all incubation times and estimated parameters. Despite of higher cell wall in Sorghum halepense in this study, the cumulative gas production was higher for it. Gas production is basically the result of fermentation of carbohydrates to acetate, propionate and butyrate and substantial changes in carbohydrate fractions were reflected by total gas produced. In addition to that this result was higher than the results obtained by (Kamalak et al., 2005) found that the gas produced at 24 hrs incubation time for alfalfa was found to be 38%. The results showed that 68.03 % of the gas for the legumes produced in the first 24 hours, while the grasses produce 67.2% of the gas in the first 24 hours of incubation. This finding is of highly interesting shows that the 24h value quoted as the most relevant value in estimating the rumen degradability rate and quality of roughages needs to be determined by gas production test. (Menke et al., 1979) found that the amount of gas which is released in 24 hrs when the feed stuffs incubated with rumen liquor is closely related to digestibility and metabolizable energy value of feed. The gas production parameters of 72 hours of incubation of the forage harvested at two times is given in table (9). For all gas production constant (a, b, a+b), late stage of growth for Rhodes grass in the pure stand and in the mixture had higher value than the early stage of growth. But for clitoria early stage of growth had a higher value than late stage of growth. The gas production of 72 hours of incubation of the forage harvested at two times is given in Table (9).

The gas produced from insoluble fraction (b) and potential gas production (a+b) for rhodes grass were higher 60 days cut for

Rhodes grass but with only significant differences, in contrast, The gas produced from insoluble fraction (b) and potential gas production (a+b) for clitoria ternata was found to be higher in 45 days cut with only significant differences in clitoria ternatea in pure stand. The rate of fermentation (c), however was increased with the advance of ripening in all forages but with significant differences only between the grasses and the legumes ($P < 0.05$). It was clear that the cumulative gas production of Rhodes grass and its association with legumes was lower than clitoria ternate, so the estimated parameter ((a, b, c and a+b) was also lower. Same results were reported by (Kamalak et al., 2005). the gas produced from slowly degradable fraction and potential gas production were lower than that reported by (Kamalak et al., 2005) For alfalfa. The potential gas production a+b for corn forage was found to be 72.6 % (Bayatkouhsar et al., 2012)

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