IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 15, Issue 11 Ser. I (November 2022), PP 01-07
www.iosrjournals.org

In Vitroand In situGas Production Technique, Ammonia (NH_3) and Volatile Fatty Acids of GrowiaTenax- Original Paper

Mohamed. A. M. ELsiddig*, Ibrahim Ahmed Bashir Ibrahim,Ayman Osman Ahmed Omar
Faculty of Animal Production, University of Gezira, Gezira, Al-Managil, P.O 20, Sudan.
**Corresponding author: elkhairey11@yahoo.com*

Abstract
Nutritive value and fermentation characteristics of leaves, green shoots, and seed cake of Grewiatenaxwere evaluated by measuring the gas production in situ for a period of 96 h. For in situ technique duplicate nylon bags were incubated for 0,2,4,6,12,24,36,48,72 and 96 h in three bults fitted with rumen cannulae. The rate of gas production (c) varied significantly (P<0.05) among the fruits and the other parts of Growiatenax. While no significant differences were observed among the green shoots, seeds and leaves. The highest rate of gas production was obtained from fruits while the lowest values were observed in the leaves. The potential degradability (a+b) varied significantly between the leaves and the other parts of Growiatenax.Generally the ammonia concentration increased with the time past feedings. No significant variations were observed between the treatments.Ruminal volatile fatty acid concentration (VFA) in this study was not significantly affected by addition of different levels of Grewiatenax fruits in to ruminant ration at levels 5%, 10% and 15%, fixed equal percentage of groundnut cake concentration. This result suggests that the Growiatenaxunder study are all potential sources of energy for ruminant animals.

Key words: gas production, Nutritive value, Ruminant, Growiatenax, volatile fatty acid

Date of Submission: 27-10-2022 Date of Acceptance: 07-11-2022

I. Introduction

Animal feed contributes more than 70 % of production costs [**1**]. Shrubs and trees are reported to be sustainable for ruminants in the tropics and subtropical areas **[2].**In Sudan, *Grewiatenax*is distributed in the dry grass savanna, in Red Sea Hills, Northern and Central Sudan, Blue Nile, White Nile, Kassala, Kordofan**[3].**In general, *Grewia* species are found to be widely distributed in the tropical and subtropical areas of Africa, Southeast Asian continents, Arabian Peninsula, Australia [**4**].also, *Grewia* species are found to be widely distributed in the tropical and subtropical areas of Africa, Southeast Asian continents, Arabian Peninsula, Australia (**5; 4**).Thus, better utilization of non-agricultural by-products which do not compete with human foods is imperative.

Young leaves of *grewiatenax* are consumed by livestock; they are slightly palatable at the end of dry seasons, and have fairly good feed value [**6]**.*Grewiatenax* is used as medicine to treat various diseases including jaundice and hepatic disorders **[7].** In Kenya plant parts are used as a remedy for colds and chest complaints and also as a chief constituent in a typhoid remedy. Also A mucilaginous bark preparation is used by women against hair vermin **[6].**

Grewiatenax common plant species are available in all seasons in semi desert area and potentially important as forage sources for honeybee. Seed dormancy is a typical feature of Grewiatenax for seed survival under unfavorable climatic conditions **[8].**Many such plants have been identified, but the lack of data on their chemical composition has limited the prospects for their utilization **[9; 10].**

The *in vitro* gas production technique measures thevolume of gas produced, which reflects the end result of thefermentation of the feed substrate to volatile fatty acids (VFAs), microbial biomass and the neutralization of the VFAs produced.Determine the nutritive value of feedstuffs, since the degradation rate and extent and rumen fermentation can be easily determined by measuring cumulative gas production **[11].** The in vitro gas production system helps to better quantify nutrient utilization, and its accuracy in describing digestibility in animals has been validated in numerous experiments **[12].**

The main objective of this study was to assess the feeding values of green shoots,leaves,flesh fruit and seeds cake of *Grewiatenax*using *in vitro* gas production technique.

The objectives of this study are to assess and study the potential macro and micro-nutrients and chemical contents of guddaim fruits, and its contents of some vitamins, amino acids, which can be considered an important for human health and the knowledge the nutritional value of guddaim fruits and to take advantage of them in various nutritional applications.

II. Materials and Methods

Study area

This study was conducted in the SudanUniversity of Science and Technology, dairy farm at kuku during November 2012.

Growiatenax tree

Growiatenax an ever green tree is multipurpose tree know for many uses

Sample collection

The *Growiatenax* ripened fruit were collected from local markets in kordofan and Darfur. The leaves and green shoot were collected manually from the tree. The seed cake was prepared by mechanical extraction of the ripened fruit.

Animal management and feeding

Three castrated steers local breed (Kenana) aged 3 years were fitted with rumen cannula as described by **[13].** They maintained and a well-balancedrations of concentrates and roughage and fed twice daily. Water and salt licks were available all time.

Ration ingredients

Ingredients of experimental ration and chemical composition as %

The experimental ration described by**Basheir, *et al.*, (14).**

Ingredients %	treatments			
	0%	5%	10%	15%
Sorghum	30	30	30	30
Wheat bran	39	34	29	24
Molasses	15	15	15	15
Groundnut cake	15	15	15	15
Growiatenax fruit	0	5	10	15
Salts	1	1	1	1
Total	100	100	100	100
Analysis				
M E (MJ/Kg)	11.11	11.68	11.04	11.01
C P%	17.30	16.80	16.47	16.05

M E= metabolisable energy; CP = crud protein; MJ = Mega joule ; Kg = Kilogram

Rumen environment

The three fistulae calves were penned with free access to water and feed, and it were fed on maintenance level on the four treatments individually.

The rumen liquor was taken by using 20cc syringe and put in clean aseptic tube from the three calves at different five periods (0,2, 4, 6 and 8 hours) for each treatment. To study the following parameters

Rumen pH reading

A sample from rumen liquor should be removed by using 20cc syringe of approximately 60 ml of rumen fluid was withdrawn from the rumen. Before taking reading the electronic pH meter should calibrate then the rumen liquor was held in container and the pH meter was immersed in the container shaken well until the reading stabilized in the pH meter and the rumen pH was recorded.

Gas production

Production of gas was determined according to the procedure of **[15].** About 200 mg of feed sample from each *Grewiatenax*tree parts was weighed in three replications and carried into calibrated glass syringes (100 ml - pre-warmed), fitted with Vaseline lubricated pistons. The inoculums used was the ruminal content of three steers with cannula in the rumen, fed free choice feed, water and mineral salts. Manually vacuum pump was used to collect rumen fluid and immediately transferred into prewar med thermos flasks to the laboratory, filtered through eight layers of cheesecloth. Buffered mineral solution was added to rumen fluid with constant stirring, while maintained in a water bath at 39°C. A total of 30 ml incubation medium consisting of 10 ml rumen fluid, 5 ml of bicarbonate buffer, 5 ml of macro-mineral solution and 10 ml of distilled water was then dispensed into pre-warmed glass syringes containing the feed samples. After closing the clips on the silicon tube at the syringe tip, syringes were gently shaken and the clips were opened to remove gas by pushing the piston

upwards to achieve complete gas removal. After closing the clip, the initial volume was recorded and the syringes were placed in a temperature controlled incubation rotor set at 39°C.

Three blanks containing 30 ml of medium as well as triplicate samples of reference hay and concentrate feed of known gas production parameters were also included as standards. Incubation was completed in duplicate within each run and runs were replicated yielding four observations per sample. Gas production was recorded before incubation (0) and 3, 6, 12, 24, 48, 72 and 96 h after incubation according to time pattern of **[16].** The gas produced due to fermentation of substrate was corrected by the blank syringes (containing no substrate). Cumulative gas production data were fitted to the model of **[17].**

Y =a+b (1-exp (-ct)

where, Y is the volume of gas produced at time „t" (ml); a is the gas production from the immediately soluble fraction (ml); b is the gas production from the in soluble fraction.; (a+b) the potential gas production (ml), and c is the gas production ratio constant for in soluble fraction (b), (ml/hr); and t is the incubation time (h).

Ammonia (NH3) determination

As described by **Conway, [18]** using Conway units. In the outer chamber of each unit 2 ml of saturated potassium carbonate (K_2CO_3)were put while in the inner chamber 2 ml of mixed indicator were pipette (40g. Boric acid + (0.02g Methylred + 0.06g.Bcg in 100 ml ethanol) complete to 2000 ml with.W.D). Covers were then replaced leaving small opening on the upper side of each unit through which 0.5 ml of the sample was added by 0.05 ml pipette.

The opening was then closed and the tilted unit set upright while shaking gently 2-3 ties so as to ensure the thorough mixing of the sample with the saturated potassium carbonate in the outer chamber.

The units were left for 6 hrs., or 40 minutes at 60c. weights were put on the top of each cover and the units were then carefully removed and the content of the inner chamber calculated against 0.01 N sulphuric acid solutions using Conway micro burette, then NH_3 in rumen liquor = T × N× 14×100 = MG /100 ml volume of the sample where

T = Titration

N= Normality of acid.

Volatile fatty aciddetermination

For volatile fatty acid determination as described by **[19]** the strained rumen liquor was deproteinizd by adding 10 ml of 0.1 normal Hcl to 10 ml from the sample 50 ml volumetric flask was the shaken thoroughly and filled to the mark with distilled water after a lapse of 5 minutes. The precipitate portion was the filtrated. 5 ml from ortrophosphoric acid was added.

The distillation was continued until 50 ml. distillations were collected in 100ml conical flask receiver. The distillation was then recovered and 3 drops of 0.04 %phenol red indicator was added. Then the nitrogen was bubbled through the distillate to remove any carbon dioxide and then titration was made against 0.01 sodium hydroxide. From a burette the amount of mill equivalents VFAs in 100 ml of sample were calculated in the following manner = V ×N×100.

Where

V = volume of NaOH

N= normality of NaOH

Statistical analysis

Data on nutrient compositions including calculated and estimated gas production parameters were subjected to one-way (ANOVA) analysis by using SAS software package [**20**] and differences of means were separated by Duncan multiple range test. Moreover, OMD and ME values using the General Linear Model (GLM) according of statistic, (Analytical software.2000).

III. Results And Discussion

Rumen PH

Rumen pH values of the animals fed the experimental diets are shown in table (2). No significant differences were observed between the control diet and the other diets or among the diets b, c and d.Animals on the control diet showed numerically lower values at all post feeding time than the other animals.

Addition adifferent levels of *Grewia tenax* fruits into the ruminant ration at levels 5, 10, and 15 % to fixed similar percentage of groundnut cake was not found to affected rumen PH up to eight hours postfeeding, PHranged from 5.05 to 6.00 in this study, this result is on line with the normalrumen pH that fund by [**21**].

The mean PH of the rumen liquor was almost the same on the test diets (6.00, 5.90 and 5.93), but was higher ($p<0.05$) for the control diet . rumen content PH was found to decrease after feeding , reaching its lowest value about 6hrs after feeding , this accord with results reported by **[22** and **23]** who determined that rumen content

PH after feeding changes in a characteristic way. There was no difference ($p<0.05$) in PH of the rumen isonitrogenous diets based on different natural protein source [**24**].
The optimal PH of rumen protiolytic enzyme renges from 5.5 to 7.0 according to **Kopecny and Wallance, [25]**; however, protein degradation was reduced as PH decreased . [**26].**

Table (2) *In situ*Rumen pH (Mean± SD) in the different incubation time (0-8 hrs) of the cattle fed different level of *Growiatenax* fruits with (SD) standard deviation.

G. tenax fruit (%) in ration	Sample fitted values of rumen pH				
	pH (0)	pH (2)	pH (4)	pH (6)	pH (8)
0	5.35±0.35^{a}	5.40±0.42^{a}	5.50±0.57^{a}	5.10±0.14^{b}	5.60±0.24^{a}
5	6.00±0.14^{a}	5.05±0.21^{a}	5.40±0.14^{a}	5.35±0.07^{ab}	5.55±0.35^{a}
10	5.60±0.28^{a}	5.05±0.21^{a}	5.55±0.07^{a}	5.35±0.07^{ab}	5.25±0.21^{a}
15	5.90±0.28^{a}	5.05±0.35^{a}	5.40±0.28^{a}	5.45±0.07^{b}	5.50±0.14^{a}
Sig	NS	NS	NS	NS	NS

a, b, c, and d : means within the same row followed by the different superscripts are significantly different ($p<0.05$)., Zero = diet content groundnut cake (control diet). , NS = not significant., SD = standard deviation.

GaGas production for the Different *Growiatenax*parts:

Gas production for the different parts of *Growiatenax*at different incubation periods is shown in the figure (1) There are considerable variations in gas production rate at all incubation times. The highest gas production was observed in the fruits, while the leaves showed the lowest gas production during all the incubation times.

Table (3) shows that the soluble fraction (a) varied significantly between the green shoots and seed cake, while no significant difference were observed between the leaves and fruits. The highest soluble fraction was obtained in the green shoots, while the lowest value in the seed.

Gas production from the slowly degradable fraction (b) varied significantly between the leaves and other parts. While no significant differences were observed between the Green shoots, fruits and seed.

Rate of gas production (c) varied significantly ($P<0.05$) among the fruits and the other parts of *Growiatenax*. While no significant differences were observed among the green shoots, seeds and leaves. The highest rate of gas production was obtained from fruits while the lowest values were observed in the leaves. The potential degradability (a+b) varied significantly between the leaves and the other parts of *Growiatenax*. While no significant difference were observed among the other parts. The highest potential degradability was obtained from the leaves while the lowest value was observed in the fruits.Organic matter digestibility (OMD) varied significantly between the fruits and the other parts of *Growiatenax*. While no significant difference was observed between the green shoots, seeds and the leaves. The lowest value was observed in the leaves.

The calculated Metabolisable energy (ME) content in the seeds cake was significantly lowest than the other parts. The leaves, green shoots and fruits have similar Metabolisable energy value.

In this study Gas production value of *Grewia tenax* leaves was 2.5, 3.5, 4, 6, 7.5 and 13.5 ml/200mg at 3, 6, 12, 24, 48, and 72 hours incubation time respectively, this result showed lower gas production value than that reported by **[27]** in the *Quereus cerris* leaves (20, 24, 35, 42, 46, and 48 ml/200mg at 3, 6, 12, 24, 48, and 72 hrs incubation time . gas production from quickly soluble fraction (a) degradable fraction (b) of*Grewia tenax* leaves in this study were 1.73% and 53.05%which was similer to the *Quereus cerris* leaves (2.40% and 50.32%) [**27**]. Gas production value of *Grewia tenax* seed cake was 3, 4, 6, 14, 17.5 and 21.5ml/200mg at 3, 6, 12, 24, 48 and 72 hours incubation time respectivly which was lower than the gas production value of *Bambara ground* seed (46, 55, 70, 91, 100 and 103ml/200mg at 3, 6, 12, 24, 48, and 72hrs incubation time respectivly[**28**]. Gas production from quickly soluble fraction (a), degradable fraction (b) and the rate of the gas production of *Grewia tenax* seed cake (c) were (0.03%, 24.04%, and 0.03%) which was lower than the 5.76%, 60.31%, and 6.23% for *Bambara ground* seeds [**28**].

Organic matter digestibility of seed cake in this study was 47.58%which was lower than the organic matter digestibility of *Bambara ground* seeds (62.72%) [**28**], it was similar to groundnut cake 48.47%, [**29**]. Metabolisable energy (ME) of the *Grewia tenax* seed cake was 4.89mj/kg DM is lower than the ME in *Bambara ground* seed 10.78mj/kgDM **[28],** groundnut cake 11.07mj/kgDM [**29**].

Rumen ammonia (NH_3)

Table (4).Instates therumen ammonia (NH_3) concentration of the animals fed the experimental diets at different times post feeding. Generally the ammonia concentration increased with the time past feedings. No significant variations were observed between the treatments, but the trend is that the control diet showed the lowest ammonia concentration, while animals on diet B and C showed the highest ammonia NH_3 concentration.

Rumen ammonia (NH_3) concentration in this study was not significantly affected by addition of different levels of *Grewiatenax* fruits into ruminant ration at levels 5%, 10%, and 15% fixed equal percentage of groundnut cake in the present studies.

Rumen content of ammonia was found to be decreased after feeding reached its lowest value about 8hrs after feeding ,**[30]** reported that decrease in ruminal PH from 6.5 to 5.7; reduced rumenal ammonia concentration, the rumen NH_3 often veries widely throughout the day depending on the feeding regime and feed quality, [**31**].

A decrease in the normal PH induced by the formation of VFA, from starch caused a decrease in the rate of NH_3liberation and an increase in protein biosynthesis [**32**]. The NH_3 absorbed from the digestive tract is very efficiently converted by the liver in to urea and should there give a response in the concentration of the blood urea [**33**].

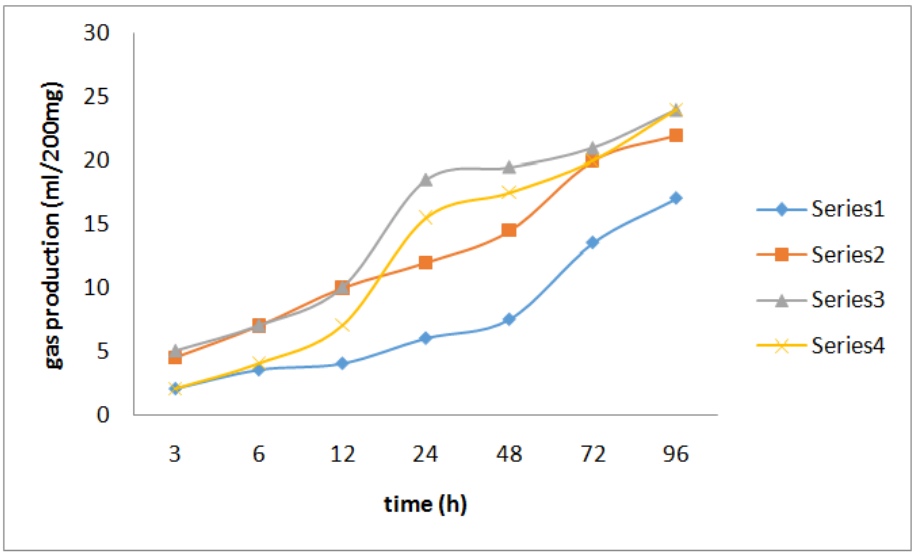

Figure (1):-
Gas production (ml/200mgDM) of experimental samples at different
Incubation times:
Series 1 = Leaves of *Growiatenax*.
Series 2 = green shoots of *Growiatenax.*
Series 3=fruits of *Growiatenax*.
Series 4 = seeds cake of *Growiatenax*.

Table (3)*In situ*Gas production kinetics of DM different experimental samples**:**

Samples	A	B	c	a+b	OMD	ME(Mj/kg)
Leaves	1.73±0.66^{b}	53.05±15.37^{a}	0.00±0.00^{b}	54.78±14.91^{a}	43.57±0.39^{c}	5.95±0.06^{a}
Green shoots	4.15±1.06^{a}	25.45±5.55^{b}	0.02±0.01^{b}	29.61±6.60^{b}	45.71±0.59^{b}	5.91±0.16^{a}
Fruit	1.11±1.26^{b}	22.22±2.20^{b}	0.05±0.01^{a}	23.33+1.17^{b}	54.39±0.78^{a}	5.51±0.12^{a}
Seeds	0.03±0.67^{b}	24.04±1.06^{b}	0.03±0.01^{ab}	24.06±2.25^{b}	47.58±0.78^{b}	4.89±0.17^{a}
Sig	*	*	*	*	*	*

a, b, c and dMeans in the same column values have different superscripts are significantly different ($p<0.05$
* = Significant.,a = the gas production from the immediately soluble fraction (ml)., b = the gas production from the insoluble fraction (ml)., c = the gas production rate constant from the insoluble fraction (b).,a+b = Potential gas production., ME =Metabolisable energy., OMD = organic matter digestibility., SEM = standard error of means.

Table (4) *In situ*rumen ammonia (NH_3) (mg/100) concentration (Mean± SD) in the different incubation time (0-8 hrs) of the cattle fed different level of *Growiatenax* fruits with (SD) standard deviation.

G. tenax fruit (%) in ration	Sample fitted values of rumen NH3				
	NH3 (0)	NH3 (2)	NH3 (4)	NH3 (6)	NH3 (8)
0	0.49±0.14	0.68±0.14	0.82±0.14	1.00±0.00	1.27±0.07
5	0.49±0.07	0.68±0.00	0.81±0.07	1.00±0.00	1.28±0.07
10	0.49±0.14	0.67±0.07	0.78±0.07	1.00±0.00	1.27±0.07
15	0.49±0.07	0.67±0.00	0.78±0.21	1.00±0.00	1.28±0.14
Sig	NS	NS	NS	NS	NS

a, b, c, and d : means within the same row followed by the different superscripts are significantly different ($p<0.05$)., Zero = diet content groundnut cake (control diet)., NS = not significant., SD = standard deviation.

Volatile fatty acids (VFAs) production

Volatile fatty acids (VFAs) concentration of different levels of *Growiatenax*cake in steer diets was shown in Table (5). The Volatile fatty acids (VFAs) concentration in this study shows no significant difference among the tested diet. Volatile fatty acids (VFAs) concentration increases with the time of incubation.

Ruminal Volatile fatty acid concentration (VFA) in this study was not significantly affected by addition of different levels of *Grewiatenax* fruits in to ruminant ration at levels 5%, 10% and 15%, fixed equal percentage of groundnut cake concentration , the VFA were highest at 8hrs post feeding and then declined slowly up to 36hrs post feeding .

Conversely, **[34]** who demonstrated VFA in rumen of sheep before feeding (4.4±0.9) ; (4.5±0.7) and 3hrs after (7.1±1.3);(7.7±1.6)and (7.6±1.3)mEq/dl. The effect of PH on VFA absorption influenceabsorption of NH_3. **[35],** reported that when rumen PH was 7.55, 6.21, or 7.58 NH_3 absorption was 26.5 and 11mEq/liter/hour and VFA was 4.18 and 4mEq/liter/hour respectively.

[36]Found that at PH on 6.5 that the transport of NH_3across the rumen epithelium increased with the concentration gradient and that NH_3 absorption was increased by absorption of VFA. Absorption of NH_3 was rapid at PH 6.5 but Negligible at PH 4.5.

Table (5)*In situ*volatile fatty acids concentration (VFA) (mg/100 ml) (Mean± SD) in the different incubation time (0-8 hrs) of the cattle fed different levels of *Growiatenax* fruits with (SD) standard deviation.

G. G. tenax fruit (%) in ration	Sample fitted values of rumen VFA				
	VFA(0)	VFA (2)	VFA (4)	VFA (6)	VFA (8)
0	0.21±0.00	0.39±0.14	0.71±0.14	0.89±0.07	1.65±0.07
5	0.20±0.00	0.39±0.14	0.72±0.07	0.88±0.07	1.65±0.07
10	0.21±0.00	0.41±0.07	0.70±0.14	0.88±0.07	1.65±0.07
15	0.20±0.07	0.40±0.00	0.69±0.07	0. 88±0.07	1.75±0.07
Sig	NS	NS	NS	NS	NS

a, b, c, and d : means within the same row followed by the different superscripts are significantly different ($p<0.05$)., Zero = diet content groundnut cake (control diet)., NS = not significant., SD = standard deviation

References

[1]. **Makkar, H.P.S., Singh, B. and Kamra, D.N. (1994),** Biodegradation of tannins in oak (Quercusincana) leaves by Sporotrichumpulverulentum. Letters in Applied Microbiology, 18: 39–41. doi: 10.1111/j.1472-765X.1994.tb00795.x.

[2]. **Melesse A, Tiruneh W, Negesse T (2011).** Effects of feeding Moringastenopetala leaf meal on nutrient intake and growth performance of Rhode Island Red chicks under tropical climate. Trop. Subtrop. Agroecosyst. 14: 485-492.

[3]. **El Amin H.M. (1990).**Trees and Shrubs of the Sudan. Board of Science and Technology for International Development National Research Council. Washington D.C.

[4]. **Aboagarib, E.A.A., R. Yang, Xia Hua, and A. Siddeeg (2014).** Chemical Compositions, Nutritional Properties and Volatile Compounds of Guddaim (GrewiaTenax.Forssk) Fiori Fruits. J. Food and Nutrition Research. 2(4): 187-192. DOI:10.12691/jfnr-2-4-9.

[5]. **Gebauer, J.; Patzelt, A.; Hammer, K. and Burkett A. (2007).** First record of Grewia tenant (Forssk), Fiori in northern Oman, a valuable fruit producing shrub, Genetic Resources and Crop Evolution 54:1153-1158.

[6]. **Orwa C, MutuaA,KindtR,Jamnadass R, Simons A. (2009).**AgrorestreeDatabase's tree reference and selection guideversion 4.0 (http://www.worldagroforestry.org/af/treedb/).

[7]. **Prasad, C. V.; Rao, K. C.; Reddy, G. V.; Rani, T.S.; Yerriswamy, B. and Subha, M.C.S. (2010).**Characteristic studies of Ligno-Cellulosic fabric Grewiatenax, Journal of Natural Fibers, 7 suppl 3: 194-215.

[8]. **Sohail, M.; Saied, A.; Gebauer, J. and Buerkert, A. (2009).** Effects of Seed Stratification Treatments on Germination of Grewiatenax(Forssk.)Fiori., a Wild Fruit Species, In: Tropentag conference on Biophysical and Socio-economic Frame Conditions for the Sustainable Management of Natural Resources.

[9]. **Vijayakumari K, Siddhuraju P. Janardhanan K. (1994).**Nutritional assessment and chemical composition of the lesser known tree legume, Acacia leucophloea. Food Chem;50:2858. 12.

[10]. **Viano J. Masotti V, Gaydou EM, Bourreil PJL, Ghiglione, Giraud M. (1995).**Compositional characteristics of 10 wild plant legumes from Mediterranean French pastures. J Agric Food Chem;43:680-3.

[11]. **Chumpawadee, S., Chantiratikul, A. and Chantiratikul, P. (2007).** Chemical composition and nutritional evaluation of protein feeds for ruminants using an in vitro gas production technique. Journal of Agricultural Technology 3(2): 191-202.

[12]. **Taphizadeh, A., A. Palangi and A. Safamehr. (2008).**Determining nutritive value of Alfalfa cuts using in situ and gas production techniques. Am. J. Anim. Vet. Sci. 3(3):85-90.

[13]. **Brown, G. F.; Armestrong and D. G.; Macrae, J.C. (1968).** The establishment is one operation of cannula into rumen and re-entrant cannula into the duodenum and ileum of the sheep. Br. Vet. J. 124,78-81.

[14]. **Basheir, E. 1, Shamseldein, H. Ahmed and Shadia A. Omer .(2015).**Degradability Characteristics of Dry Matter and Crude Protein of Growiatenax Using the in situ and in vitro Techniques in Ruminants -Sudan Journal of Science and Technology, 16(1): 68-75.

[15]. **Menke, K. H. Raab, L. and Schneider, W. (1979).**The estimation of digestibility and metabollizable energy content of ruminant feedstuff from the gas production when they incubated with rumen liquor in vitro .J .of Agric. Sci. (Cambridge).92:217-222.

[16]. **Blummel, M. and Qrskov ER (1993).** Comparison of in vitro gas production and nylon bag degradability of roughages in predicting feed intake in cattle. Animal feed science and technology 40: 109-119.

[17]. **Qrskove E. R and McDonal, (1979).** The estimation of protein degradability in the rumen from incubation measurements weighed according to rate of passage. J.Agric.sci 92:499-503.

[18]. **Conway, E. J. (1657).**Microdiffusion analysis and Volumetric Error 4thed,London, Crosby, Lock-wood and Son Ltd.

[19]. **Kroman RP, Meyer JH and Stielau WJ (1967).**Steam distillation of volatile fatty acids in rumen digesta. Journal of Dairy Science, 50: 73-76.

[20]. **SAS, Statistical Analysis System (2004).**Ver. 9.0.SAS Institute Inc. Cary North California, USA.

[21]. **Owens, F. N., Secrist, D. S., Hill, W. J. and Gill, D. R. (1998).** Acidosis in cattle: a review. J. Anim. Sci. 76: 275-286.

[22]. **Fenner, H., Dickinsonf. N., Bames H.D.(1967)**.Relationship of digestibility and certain rumen fluid components to level of feed intake and time of sampling after feeding, jDairySci; 50.334-344.

[23]. **Prakash, P. Reddy, D. V. Ramachandra R. Reddy, N. Krishna. (1996).** The catalytic effect supplementation of protein meals on utilization of rice straw-poultry droppings-rice bran diet in buffaloes Animal feed Science and technology, Volume 63, Issues 1-4, 1 December 1996, page 229- 243.

[24]. **Sehgal, J. P.; singh, M.; Rawat, P. S. and Singh. R. N. (1984).** Feedlot performance of growing lambs for mutton production. Ind. J. Ani. Sci. (1983) (7): 715 – 719. Arikanagar, Rajasthan, India.

[25]. **Kopecny, J. and R. J. Wallace. (1982).** Cellular location and some properties of proteolytic enzymes of rumen bacteria. Appl. Environ. Microbiol. 43, 1026-1033.

[26]. **Cardozo, P. W., S. Calsamiglia, and A. Ferret. (2002).**Effects of pH on microbial fermentation in high concentrate diets in a dual flow continuous culture system. J. Dairy Sci. 85(Suppl. 1):182. (Abstr.)

[27]. **Conbolat, O. Kamalak, A. Ozkose, C. O. Sahin, M. and Karabay, (2005).**Effecr of polyethylene glyeoloninvitro gas production metabolizable energy and organic matter digestibility of quercuscerris leaves. Feed Sci. technol , 81:2-22.

[28]. **Abdallah. A. (2007).**Nutritive evaluation of Bambara groundnut (Vignasublerranean) pods and seeds and Holl as animal feeds.M.V.Sc thesis, University of Khartoum – Sudan.

[29]. **Menke, K. H. and H. Steingass. (1988).** Estimation of energetic feed value obtained from chemical analysis and gas production using rumen fluid. Anim Res. Development, 28: 7- 55.

[30]. **Lana, R. P. J. B. Russell, and M. E. Van Amburgh, (1998)**. The role of PH in regulating ruminal methane and ammonia production. J. amin.Sci 79: 2190-2196.

[31]. **Tiwari,C. M. A. S. Chandramonil, S. B. Jadhao2, S. K. Gowda and M. Y. Khan, (2001).** Studies on blood chemical constituents and rumen fermentation in growing buffalo calves fed ammoniated straw-based rations supplemented with different protein sources Available online 9 February 2001. Izatnagar, 243 122, India.

[32]. **Reis, P. J and Reid, R. L (1959).**In vitro studies on the effect of pH and of glucose on Ammonia accumulation in the rumen sheep. Aust. J. Agric Res 10; 71 -80

[33]. **Cizuk, P. and Eriksson, S. (1973).**Ammonia fermentation in the rumen of sheep fed on grass, clover or Lucerne presented in various ways. Swed. J. Agric. Rec. 3: 13-19.

[34]. **Briggs, P. K. Hogan, J. P. and Reid, R. L. (1957).**The effect of volatile fatty acid, lactic acidand ammonia in rumen PH in sheep. Aust, J. Agric, Res, 8: 674- 690.

[35]. **Bloomfield, R.A., Kearley, E O., creach, D.O and Hurer, H.E (1963).**Ruminal PH and absorption of ammonia and VFA.J Anim Sci., 22,833 (Abstr).

[36]. **Khemiss, F.; Ghoul-Mazgar, S.; Moshtaghie, A. A. and Saidane, D. (2006).**Study of the effect of aqueous extract of Grewiatenax fruit on iron absorption by everted gut sac, Journal of Ethnopharmacology, 103Suppl 1: 90-98.

Mohamed. A. M. ELsiddig, et. al. "In Vitroand In situGas Production Technique, Ammonia (NH3) and Volatile Fatty Acids of GrowiaTenax- Original Paper." *IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS),* 15(11), 2022, pp. 01-07.