Online Journal of Animal and Feed Research

Volume 6, Issue 3: 62-72; May 30, 2016



IN VITRO DIGESTIBILITY OF SELECTED FORAGES IN SARGODHA DISTRICT, PAKISTAN

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ABSTRACT: The present study conducted to evaluate the digestibility values of four tree fodder species i.e. Mulberry (*Morus alba*), Kikar, (*Acacia nilotica*), Ber (*Zizphus jujube*) and Shirin (*Albezia procera*); three grasses i.e. Bermuda grass (*Cynodon dactylon*), Mott grass (*Penisetum perpureum*) and Rhode grass (*Chloris gayana*) and two fodder crops i.e. Sorghum (*Sorghum bicolor*) and Alfalfa (*Medicago sativa*) were selected as treatment having three replicates. Duplicate sample of each treatment was collected from seven sub districts of Sargodha. The results showed that dry matter content varied from 17.50% in *Penisetum perpureum* to 44.23% in *Albezia procera*. Crude protein contents were highest in *Morus alba* (22.56 %) and lowest in *Sorghum bicolour* (5.60 %). Acid detergent insoluble fiber (ADF) and neutral detergent insoluble fiber (NDF) values were highest for *Penisetum perpureum* (ADF 45.43%and NDF 74.56%) and lowest for *Acacia nilotica* and *Zizphus jujube* (ADF 14.46% and NDF 31.56%), respectively. The ash contents were highest in *Penisetum perpureum* (11.50%) and lowest in *Cynodon dactylon* (5.46%). *In vitro* DM digestibility was determined at different time intervals (6, 12, 24 and 36 hours) and found highest P<0.05; 78.26% in *Morus alba* and lowest 54.20% in *Chloris gayana*. In conclusion, results recommended that the *Morus alba forage* use as alternative cheap source of ruminants due to high nutritive and IVDMD (*In vitro* dry matter digestibility) values.

pii: S222877011600009-6 Received 06 Mar. 2016 Accepted 15 Apr. 2016

Key Words: Forages, Digestibility Evaluation, Ruminants

INTRODUCTION

Livestock contributes approximately 55.9 percent value addition in agriculture and 11.8 percent of national GDP in Pakistan (Economic survey of Pakistan, 2013-14). Livestock addition to the gross value has increased from Rs.756.3 billion (2012-13) to Rs.776.5 billion (2013-14). That shows the progress of 2.7 percent as compared to previous year. There are 39.7 million cattle, 34.6 million buffalo, 29.1 million sheep, 66.6 million goats, 1.0 million camels, 0.4 million horses, 4.9 million asses and 0.2 million mules in Pakistan (Economic survey of Pakistan, 2014) out of which Sargodha has about 696 thousand cattle, 799 thousand buffalo, 143 thousand sheep and 675 thousand goats. Production per animal is very poor as compared to others countries and factor behind this situation is shortage of nutrients (Sarwar et al., 2002). Livestock require 13.5 and 10.3 million tons of CP and TDN, respectively (Economic survey of Pakistan, 2006). Present feed resources fulfil only 62% CP and 75% TDN requirement of livestock. Tree leaves and grasses could be used as a feed complement and they provide upper limit dietary profit (Bhatta et al., 2005). Fodder crops cover only 14% total area cultivated in the country and are decreasing with the passage of time as 2% every 10 years due to high demands of cash crop production (Gill, 1998). Forages are main component of feed for dairy cows on the grounds that they give coarse fiber to improve rumen capacity and they must be supplemented with other ingredients. Forages have been generally examined for CP and fiber fixations because of contribution in the formulation of feed (Masahito and Mike, 2005). Grasses, foliage of trees, shrubs and water plants are major components of ruminants feed (Wanapat et al., 2008). There are several alternatives for improving the performance of ruminants by providing low quality basal diets. One of the alternatives is to use trees and shrubs which are high in protein content but have moderate to high digestibility (Egan, 1997). Recently, in many tropical countries and regions, there has been a focus on identifying and using locally available shrubs and tree leaves as a source of feeds or feed ingredients for ruminants because of their high nutritive value and positive effects on rumen function (Omar et al., 1999; Yao et al., 2000). However, due to its high protein content with which quality is comparable or even superior to that of soybean meal (Nguyen Xuan Ba et al., 2005).

To cite this paper: Arif M, Hayat Z, Saeed M, Asif Arain M, Ali Shah Q, Ali Siyal F, Faizi Z, Soomro RN, Abbasi IHR, Rehman A, Abbas Raza SH, Hayat F. 2016. In vitro digestibility of selected forages in Sargodha district, Pakistan. Online J. Anim. Feed Res., 6(3): 62-72. Scienceline/Journal homepages www.science-line.com www.ojafr.ir

In Pakistan green leaves of trees and some grasses are fed to the large and small ruminants in extreme climate during fodder scarcity. In those areas where limited source of fodder is available for the animals, tree leaves are provided to the animals for their nutrition (Reddy, 2006). In small ruminant protein and energy sources are obtained from tree leaves (Singh et al., 1989). Diets supplemented with these two mulberry products in an isocaloric and isonitrogenous manner have similar effects to corn grain and cotton seed meals on steer performance, blood biochemical parameters and carcass characteristics (Zhou et al 2014). Fodder tree leaves are important components of sheep and goat feed these are also provided during scarcity period because they contain proteins, vitamins and minerals (Kamalak et al., 2004). Plants can be easy and cheap source of energy for ruminants as they maintain higher total sugar content that improves the growth animals (Areghore and Hunter., 1999 and Ahmad et al., 2008).

Nutritional deficiencies in ruminants mostly occur by anti-nutritional factors such as tannins and other secondary compounds. Fodder tree legumes and grasses have high protein level, so deficiencies occur in ruminants due to anti nutritional factors such as tannins and other secondary compound that can be minimized by using as feed supplement in ruminants (EL-Waziry, 2007). Plants having anti-nutritional factor as tannins or phenol compounds can be utilized in rumen of goat by certain bacteria such as *Streptococcus caprinus*, *Selenomonas ruminantium*, *Prevotella ruminicola*, *Butyrivibrio* spp., *Lactobacillus* spp. and *Enterobacteriacae* spp. These bacteria can utilize both condensed and utilizable tannin (Pell et al., 2001). There is an imperative need for the development of rapid screening technique and proper methods to study digestibility of different forages. Keeping in view, the situation of livestock, nutrient availability and importance of different forages, in Sargodha district.

Present study was planned for the evaluation of *in vitro* digestibility estimation of different nine fodder species available in Sargodha district of Pakistan and to explore their nutritive value. The information gained would be useful in the estimation of nutritive value and digestibility of different forages in this area.

MATERIAL AND METHODS

Sampling of forage species

Based on data taken from the farmers through the questionnaire, sample of commonly available forages were taken and identified on the basis of their botanical names Table 1. One kg sample of each species was collected from two different sites of each tehsil. Composite samples were prepared and packed as sooner in polythene bag. These composite samples were taken in the laboratory of University College of agriculture, University of Sargodha. The schedule of sample collection is shown in Table 2. The samples of forages were analyzed for proximate analysis.

Chemical analysis

Chemical analysis of selected forages was noted by taking randomly, 250g sample from each bag and then it was dried in hot air oven at 65-70 °C and was stored for further analysis. The oven dried samples were ground through 1mm sieve and were analyzed for DM, CP, NDF, ADF and ASH (AOAC 1990; Van Soest et al., 1991).

Moisture and Dry matter estimation

Moisture in fecal samples was determined by drying the known quantity (W_1) of sample at 105° C in a hot air oven for 24 hours. After 24 hours, samples were transferred directly from oven to desiccators for 5-10 minutes to cool them down to and then weighed again W_2 . Moisture contents in samples were calculated using following formula:

Moisture (%) = $\frac{W_1 - W_2}{W_1} \times 100$ Where

 W_1 indicates weight of sample before drying and W_2 stands for weight of sample after drying. Dry matter was determined by using the formula: DM (%) = 100 – Moisture contents

Crude protein determination

Total nitrogen in a sample was determined by Kjeldhal method. A known amount of the oven dried sample (W_1) was taken in along Kjaldhal flask. Five grams of a catalyst mixture CuSO₄ (9:1) and 25 ml of concentrated H₂SO₄ was added the sample and boiled in a digestion rack initially at low temperature and then at vigorous boiling till the content became clear. After cooling the contents of the flask, distilled water was added in a 250 ml volumetric flask for dilution. A 10 ml of this solution was transferred to micro Kjaldhal distillation apparatus and distilled in a presence of 50 mg of zinc dust and 40 % NaOH solution. The ammonia so produced was collected in beaker containing 2 % boric acid solution having two drop of methyl red as an indicator. The distillate was titrated

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against standard 0.1 NH₂SO₄ solution to light pink indicate the percentage of nitrogen was calculated according to the following formula.

% CP = {(Vol. of 0.1N H₂SO₄ used × Dilution of sample solution × 0.0014 × 6.25) / (Weight of sample × sample solution used)} × 100.

Ash determination

A known amount of samples (W_1) was taken in a crucible. It was heated on an oxidizing flame till no smoke was given out. The crucible was then placed in muffle furnace at about 600 $^{\circ}$ C till all the oxidized matter was recorded. The percentage of ash was calculated by the following formula.

Ash % = weight of ash (W_1)

weight of sample (W₂)

W₁= Wt. of crucible including sample weight.

W₂= Wt. of sample after burning.

Neutral detergent in soluble fiber determination

One gram of dry, well-ground homogenized feed sample was taken and passed through 1mm mesh in 500 ml capacity flask and added 0.5 gm of sodium sulphite. The solution of 100 ml ND solution was added across the wall of cylinder for the prevention of soap forming. Rapidly it was brought to boiling temperature and boiled gently for an hour and watch glass was placed on the flask for condensing purpose. Then the flask was removed, cooled and filtered through suction assembly. Residue was washed with hot water (85-95 °C) to remove ND solution and then washed with acetone (20 ml) and shifted the residue in crucible and constant weight was taken by putting at 105 °C repeatedly.

NDF = (weight of crucible + cell wall contents) - weight of crucible \times 100/weight of dry sample.

Acid detergent fiber determination

Approximately 1 gram of sample was weighed into a beaker or container suitable for reflux by difference. Acid detergent solution (room temperature) of 100 ml cold and 2ml deca-hydro-nephthlene were added and heated for boiling in 5 to 10 minutes. Then heat was reduced to avoid foaming as boiling had begun. Reflux 60 minutes from onset of boiling and adjusting boiling to as low even level. It was filtered on a previously weighed crucible, that was set on the filtering apparatus using light suction and broke up the filter material with a rod and washed twice with hot water (90-100°C) crucible in the same manner. It was washed with acetone and repeated until that showed no more colour and then was broke up all the lamps so that come into contacts with all particles of fiber. Then hexane was washed and it was added while crucible still contained some acetone (hexane 8 was omitted because of lumping problem). Acid detergent fiber free of hexane was sucked and dried at 105°Covernight. Then it was cooled in desiccators at room temperature and weighed.

ADF= (Wt. of crucible+ fiber- empty wt. of crucible)

Wt of sample on dry matter bases

In-Vitro Dry matter Digestibility

The forage samples were analyzed for *in-vitro* DM digestibility according to the method as described by Tilley and Terry (1963). The fresh rumen contents were brought from local slaughter house in insulated bottles and transported immediately to the experimental site. The rumen contents were squeezed through four layer of cheese cloth kept in water bath having temperature 39 °C until incubation take place. Representative samples of the mixtures (2.5g DM) was taken in a separate bottle having 0.05 liters rumen liquor 0.2 liters buffer solution (Buffer solution: KCI 0.57 g/L, MgSO₄. 7H₂O 0.12 g/L, NaCI 0.47 g/L, CaCl₂ 0.04 g/L, Na₂HPO₄.12H₂O 9.30 g/L, NaHCO₃ 9.80 g/L, Cysteine 0.25 g/L (Elmenofy *et al.*, 2012, Tilley and Terry, 1963). The bottles were kept in water bath having fix temperature 39°C degree. The samples were run for *in-vitro* DM digestibility at 6, 12, 24 and 36 hours of incubation. The in *in-vitro* DM digestibility was determined by using following formula.

Initial weight-final weight

In-vitro DM digestibility % = -

Initial weight

Statistical analysis

The data recorded was subjected to statistical analysis using analysis of variance under CRD. The difference among treatments was studied as described by Steel et al. (1997).

Table 1 - Botanical names of different experimental forages. Sr No **Forage names Botanical names** 1 Bermuda grass Cynodon dactylon 2 Mott grass Penisetum perpureum 3 Rhode grass Chloris gayana 4 Kikar Acacia nilotica 5 Mulberry Morus alba 6 Ber Ziziphus jujube 7 Shirin Albezia procera 8 Sorghum Sorghum bicolor 9 Alfalfa Medicago sativa

Table 2 - Sample collection schedule from different tehsils of Sargodha district.						
Sr No	Location	Date	Туре			
1	Sahiwal	15-03-2014	Forages			
2	Kotmomin	17-03-2014	Forages			
3	Bhera	19-03-2014	Forages			
4	Bhalwal	21-03-2014	Forages			
5	Silanwali	23-03-2014	Forages			
6	Sargodha	25-03-2014	Forages			
7	Shahpur	27-03-2014	Forages			

RESULTS AND DISCUSSION

Dry matter

Results of present study showed that DM content in Albezia procera was the highest (P<0.05; 44.23%) and lowest for Penisetum perpureum (17.50%; Table 3). Results of this study are in line with the finding of (Datt et al., 2008) who reported that higher DM contents of Albezia procera as compared to other forage species ranging from 43.14 to 47%. The highest DM content of Albezia procera might be attributed to higher level of cell wall component and lower level of moisture contents (Weinberg and Muck, 1996) Results of present study are not in line with the finding of Borreani et al. (2007) who found higher (19.35%) DM contents in Cynodon dactylon and lower (17.02%) in Cenchrus ciliaris from spring to summer. This might be attributed to unhealthy leaves and absence of flowers in this season.

Results of the current study indicated that CP value of the *Morus alba* was the highest (P<0.05; 22.56%) while that of *Sorghum bicolor* was the lowest (5.60%; Table 3). Results of this study are same with the finding of Cheema et al. (2011); Kandylis et al. (2009) and Shayo. (1997) who reported that CP value of *Morus alba* were more as compared to others forages ranging from 18.6 to 23%. Higher CP level of *Morus alba* than other forages might be due to more accumulation of protein content in them during growth. Yulistiani et al. (2015) reported that Supplementation of mulberry to TRS-based diet at 1.2% BW or at 32% of total diet had similar effect to urea rice bran supplementation on the DMI, nutrient digestibility and N utilization that create efficient of rumen ecosystem and microbial protein supply It is reported that *Morus alba* can be used as main feed in small ruminants (Yao et al., 2000). However, present study differs with Omar et al. (1999) who reported that CP content in mulberry leaves was 15.9%. Hirano (1982) and Mandal (1997) found that CP value in mulberry leaves was lower; it might be attributed to differences in localities. Alam and Djajanigra (1994) reported that rumen degradation is affected if the level of CP in feed is less than 10 %.

Neutral detergent fiber and Acid detergent fiber

Results of the this study indicated that NDF and ADF contents of the *Penisetum perpureum* g rass were 74.56 and 45.43%, respectively while NDF contents was the lowest (31.56%) in *Ziziphus jujube* and ADF contents was minimum (26.33%) in *Sorghum bicolor*. Results of current study are in line with the finding of Sarwar and Nisa, (1999) and Touqir et al. (2009) who reported that NDF and ADF value of *Penisetum perpureum* were 70.6 and 62.0% and 40.8 and 32.4%, respectively. The result might be due to increase in amount of fiber component because of other cell content having carbon skeleton is converted into fiber component and lignin content increase (Ruiz et al., 1992; Smith et al., 1972). Higher in NDF concentration have negative effect on the performance of animals that can reduce intake of energy (Zinn and Ware, 2007). Lower values of ADF in these forages showed higher potential ruminant feed (Bakshi & Wadhwa, 2007). Results of this study differ with the finding of Liu *et al.* (2002) who reported that leaves contain high protein and low fiber as compared to stem and trunk.

Table 3 - Chemical composition of different forages

Items (%)	Cynodon dactylon ¹	Penisetum perpureum ²	Chloris gayana ³	Acacia nilotica ⁴	Morus alba⁵	Ziziphus jujube ⁶	Albezia procera ¹	Sorghum bicolor ⁸	Medicago sativa ⁹	s.e.m ²
Dry matter	30.10 ^d	17.50 ^f	40.04 ^b	33.50°	28.84 ^b	33.50ª	44.23ª	17.61 ^f	20.77°	0.32
Crude protein	12.43 ^{de}	11.46°	9.63 ^f	11.40°	22.56 ª	13.76 °	13.26 ^{cd}	5.60 ^g	16.00 ^b	0.21
Neutral detergent fiber	65.60 [⊳]	74.56ª	59.26°	23.13 ^f	23.66 ^f	31.56°	45.63 ^d	44.53 ^d	32.43°	0.28
Acid detergent fiber	29.03°	45.43ª	38.53 ⁵	14.46 ^e	14.53°	15.43°	30.50°	26.33 ^d	27.13 ^d	0.29
Ash	5.46 ^{fg}	11.50 ª	8.70 ^b	5.80 ^{ef}	8.43 ^{bc}	7.56 ^{cd}	7.33 ^d	4.56 ^g	6.70 ^{de}	0.22
^{abcdefg} Means on the same Standard error mean	rows with differen	t superscripts are sign	ificantly different(p	o<0.05) ¹ Bermuda(Grass, ² Mott grass	s, ³ Rhode grass, ⁴	Kikar⁵Mulberry, ⁶	Ber ^{, 7} Sirin, ⁸ Sorghu	ım,ºalfalfa; SEM= s	stand for

ltems (%)	Cynodon dactylon¹	Penisetum perpureum ²	Chloris gayana ³	Acacia nilotica ⁴	Morus alba ⁵	Ziziphus jujube ⁶	Albezia procera ¹	Sorghum bicolor ⁸	Medicago sativa ⁹	S.E.M
After 6 (h)	23.33e	20.10 ^f	25.43 ^d	31.46 ^b	35.00ª	23.40 ^e	25.63 ^d	29.53°	29.80°	0.14
After 12 (h)	32.53 _{cd}	31.60 _{de}	28.80 ^f	34.10 _{bc}	43.40 ª	30.63°	34.70 ^b	31.56 _{de}	34.20 _{bc}	0.48
After 24 (h)	41.70°	50.53 ⁵	32.30 ^h	41.50°	68.29ª	33.76 ^g	48.53°	38.20 ^f	42.50 ^d	0.11
After 36 (h)	53.13°	53.16°	39.46 ^d	53.36°	78.26ª	59.80 ^b	51.96°	52.36 _{ab}	51.60°	0.55

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Ash

Results of the current study indicated that ash content of *Penisetum perpureum* was the highest (P<0.05; 11.50%) and the lowest for *Sorghum bicolor* (4.56%; Table 3). Results of this study are same with the finding of Bilal (2008); Touqir et al. (2007). Arshadullah et al. (2009) who reported higher ash contents of *Penisetum perpureum* as compared to other forages and were in range from 9.60% to 12.40%. The ash content in forage may be derived from two sources i.e. internal plant source and external source such as soil. Total ash content of fodder trees, shrubs and climbers was high. The average result (8.03%) of current study is not similar with the finding of Manzoor, (2013) who reported that average ash content was 13.70%.

In vitro dry matter digestibility

Results of the current study indicate that IVDMD at 36 hours of incubation was the highest (P<0.05; 78.26%) for *Morus alba* while that of *Chloris gayana* was the lowest (39.46% Table 4). Results of this study are similar with the finding of Bakshi and Wadhwa, (2007) Omar et al. (1998); who reported that IVDMD of *Morus alba* leaves was higher than other forage species. Shayo (1997) reported IVDMD of *Morus alba*leaves was 82.1%. This might be attributed to high CP level and increased concentration of ammonia nitrogen in rumen (Hristov et al., 2004). Higher ammonia nitrogen in rumen improves microbial activity and growth of fibrolytic bacteria resulting in more DM digestibility Griswold et al. (2003). In-vivo digestibility and in-vitro of mulberry leaves was 78.4-80.8% and was 80.2-95.0% respectively (Sanchez, 2000). Maximum DM digestibility of *Morus alba* was noted due to more in CP level and less in NDF level Wiedmeier et al. (1983). Results of present study are not in line with the finding of Cheema et al. (2011) who found higher (92%) digestibility of fresh *Mous alba* leaves. This might be due to *in-situ* digestion trial on buffalo bull with fresh mulberry leaves. Dry *Morus alba* leaves had less CP value which may affect DM digestibility (Playne, 1978; Kawashima et al. 2007).

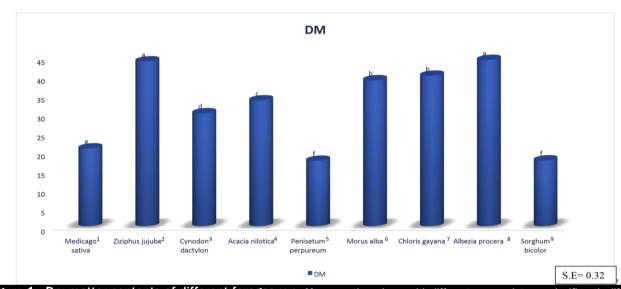


Figure 1 - Dry matter contents of different forages (abcdeg Means on the column with different superscripts are significantly different (p<0.05); ¹alfalfa. ²Ber, ³Bermuda Grass, ⁴Kikar, ⁵Mott grass, ⁶Mulberry, ⁷Rhode grass, ⁸Sirin, ⁹Sorghum; S.E. stand for Standard error mean; h: hour).

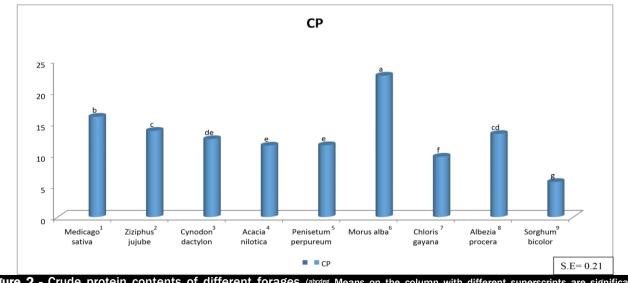


Figure 2 - Crude protein contents of different forages (abcdeg Means on the column with different superscripts are significantly different (p<0.05); ¹alfalfa. ²Ber, ³Bermuda Grass, ⁴Kikar, ⁵Mott grass, ⁶Mulberry, ⁷Rhode grass, ⁸Sirin, ⁹Sorghum; S.E. stand for Standard error mean; h: hour).

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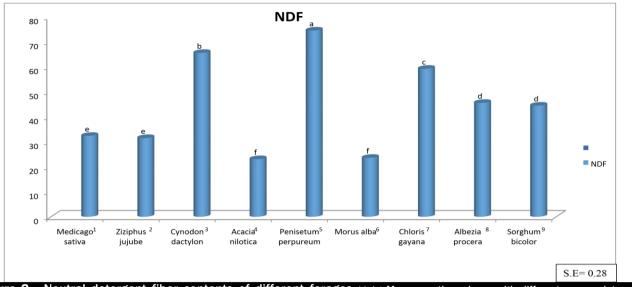


Figure 3 - Neutral detergent fiber contents of different forages (abcdeg Means on the column with different superscripts are significantly different (p<0.05); ¹alfalfa. ²Ber, ³Bermuda Grass, ⁴Kikar, ⁵Mott grass, ⁶Mulberry, ⁷Rhode grass, ⁸Sirin, ⁹Sorghum; S.E. stand for Standard error mean; h: hour).

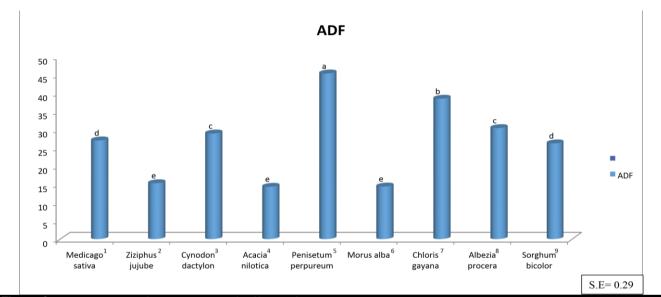
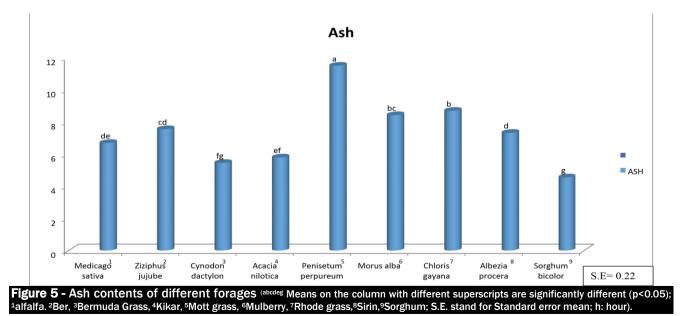


Figure 4 - Acid detergent fiber contents of different forages (abcdeg Means on the column with different superscripts are significantly different (p<0.05); ¹alfalfa. ²Ber, ³Bermuda Grass, ⁴Kikar, ⁵Mott grass, ⁶Mulberry, ⁷Rhode grass,⁸Sirin,⁹Sorghum; S.E. stand for Standard error mean; h: hour).



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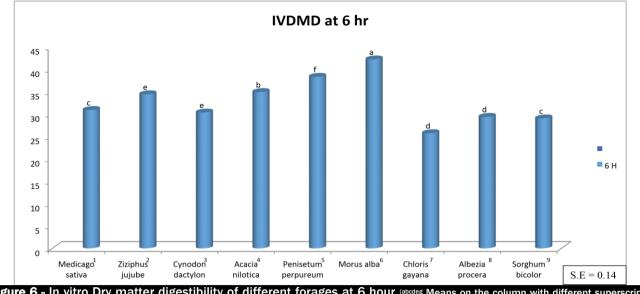
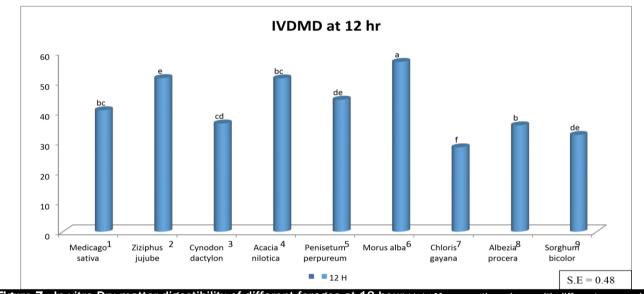
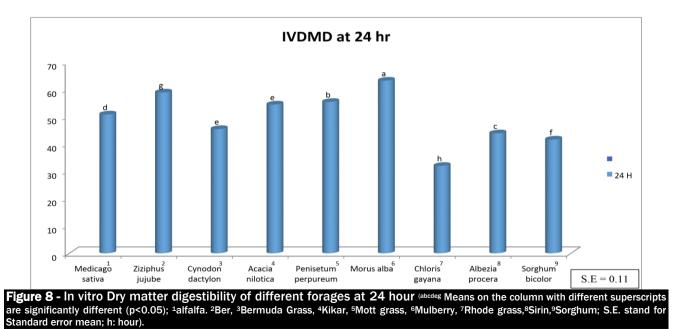


Figure 6 - In vitro Dry matter digestibility of different forages at 6 hour (abcdeg Means on the column with different superscripts are significantly different (p<0.05); ¹alfalfa. ²Ber, ³Bermuda Grass, ⁴Kikar, ⁵Mott grass, ⁶Mulberry, ⁷Rhode grass, ⁸Sirin, ⁹Sorghum; S.E. stand for Standard error mean; h: hour).

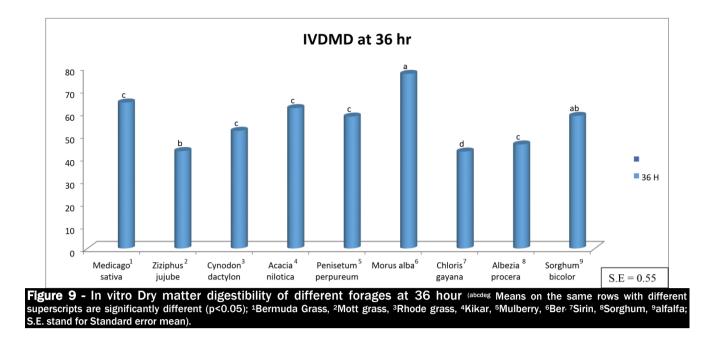






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CONCLUSION AND RECOMMENDATIONS

In conclusion, the results of our study recommended that the above mentioned forage use as alternative cheap source of nutrient so could be supplemented in ruminant feed due to high nutritive and IVDMD (*In vitro* dry matter digestibility) values.

Competing interests

The authors declare that they have no competing interests exist.

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