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# Effect of replacing alfalfa hay with *Leucaena leucocephala* (*L. Leucocephala*) leaves on *in vitro* gas production, digestibility and *in situ* degradability in buffalo

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**ABSTRACT.** This study was performed to investigate the effect of replacing alfalfa hay by *L. leucocephala* leaves in proportions of 25, 50 and 100% on *in vitro* gas production (GP) parameter, digestibility and *in situ* degradability in buffalo. Results showed that the volume of GP at 2 to 12 hours after incubation was significantly affected by replacing alfalfa hay with *L. leucocephala* leaves. *In vitro* digestibility of organic matter (OMD) differed significantly between treatment as it declined by increasing the alfalfa hay substitution rate from 25 to 100%. The microbial crude protein (MCP) differed significantly between treatments and was the greatest of 589 and 599 mg $g^{-1}$ of dry matter (DM) when *L. leucocephala* leaves replaced alfalfa hay at 25 and 50%. The *in vitro* digestibility of DM (IVDMD) increased significantly at 50% *L. leucocephala* replacement rate. Moreover, substituting alfalfa hay by *L. leucocephala* had a significant effect on the *in situ* degradability parameters. The insoluble but potentially degradable fraction (B) and potential of degradability (A+B) significantly increased for treatment contain 50% *L. leucocephala* leaves. The effective degradability (ED) was significantly different between dietary treatments and was the greatest when alfalfa hay was replaced by 25 and 50% *L. leucocephala*. In conclusion, *L. leucocephala* leaves can substitute 25 to 50% of dietary alfalfa hay in buffalo rations without effect on rumen efficiency.

**Keywords:** buffalo; *in situ* digestibility; gas production; *leucaena leucocephala* leaves.

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## Introduction

The increase in human populations lead to an increase in food demand (Choct, 1997). The magnitude of animal production is influenced by several factors, in which the role of nutrition represents a major part to ensure that nutrients are supplied to animals adequately. Nutritionists are seeking a practical and scientific solutions to improve animal productivity (Direkvandi et al., 2020). Especially in tropical and sub-tropical regions where feeds often are low crude protein (CP) and rich in crude fiber (Wanapat, 2000). Likewise, in arid areas forages like alfalfa hay is either in shortage or imported and formulated at high market prices (Alqaisi, Moraes, Ndambi, & Williams, 2019).

Substituting typically imported feeds with those of feed of native origins reduced feed cost in arid dairy feeding systems (Alqaisi, Hemme, Latacz-Lohmann, & Susenbeth, 2014). Despite of its availability and quality, one of the problems associated with feeding tropical plants to ruminants is that they contain anti-nutritional substances that reduces animal's feed efficiency. *Leucaena leucocephala* is a well-known tropical tree. *L. leucocephala* contains anti-nutritional factors such as mimosine (3-10% of dry matter (DM); Gupta & Atreja, 1999), tannins (total tannin between 2.4 and 3.3%; Islam, Nahar, & Islam, 1995), in addition to saponin, oxalate and alkaloids. The presence of condensed tannin in the *L. leucocephala* can prevent protein digestion in the rumen (Kang, Wanapat, Pakdee, Pilajun, & Cherdthong, 2012). Tannins are one of the phenolic compounds that are commonly found in some trees, shrubs, legumes as well as in some grains (Sabu, Augur, Swati, & Pandey, 2006). Tannins have anti-nutritional effects which reduces palatability, digestibility, protein availability and digestive disturbances in animals, these effects mostly resulting from the decline in rumen microflora activity by reducing the availability and digestibility of nutrients and minerals (Molan, Attwood, Min, & McNabb, 2001), cell wall destruction (O'Donovan & Brooker, 2001) and interference with extracellular enzyme activity (Frutos, Hervás, Giráldez, & Mantecón, 2004).

Despite of its anti-nutritional contents, the *L. leucocephala* is an evergreen legume contains leaves and pods that are rich in CP and neutral detergent fiber (NDF) being 235 and 386 g $kg^{-1}$ of DM, respectively (Clavero & Razz, 2003). Garcia, Ferguson, Neckles, and Archibald, (1996) reported a high CP content of *L. leucocephala* leaves and forages of 292 and 220 g $kg^{-1}$ of DM. Furthermore, *L. leucocephala* seed and leaves are a good source of minerals such as potassium, phosphorus, nitrogen, calcium, magnesium, zinc, copper and manganese (Alabi & Alausa, 2006). Feeding a mixture of *L. leucocephala* and forage increased milk production in lactation cows (Harrison, McSweeney, Tomkins, & Eckard, 2015). Moreover, supplementing a *L. leucocephala* in a total ration has increased feed intake (Islam et al., 1995). In view to its nutritional value, *L. leucocephala* might be used as a substitute feeds for ruminants in arid areas. Therefore, the objective of this study is to investigate the effect of substituting alfalfa hay with varying levels of *L. leucocephala* leaves on *in vitro* gas production (GP) parameter, digestibility and *in situ* degradability in buffalo.

## Material and methods

This study was carried out at the research farm of Agricultural Sciences and Natural Resources University of Khuzestan (Mollasani, Iran).

### Forage preparation and experimental diets

The *L. leucocephala* leaves were prepared from *L. leucocephala* trees available on Mollasani city (Iran, Khuzestan). Sampling was performed by plot method from 10 trees. Then the equal amount of samples mixed, air-dried in the shadow and grounded. Leaves were then chopped into approximately 20 mm particle length and DM was evaluated. Alfalfa hay was grown in Iran on irrigated land and was supplied to the university farm monthly. Alfalfa hay samples were collected from the university farm. In this experiment, alfalfa hay was replaced by *L. leucocephala* leaves in proportions of 0 (without *L. leucocephala*; control), 25, 50 and 100%.

The diets were formulated according to National Research Council (NRC, 2001) and were homogenized in their metabolizable energy (ME) and CP contents. The control diet reflects a typical feed ration offered to buffalo cattle in Iran. Table 1 shows the ingredients and the chemical composition of the experimental diets used for the *in vitro* GP, digestibility and the *in situ* DM degradability.

The contents of DM, Ash, CP, NDF, ether extract (EE) and total tannin (TT) of *L. leucocephala* leaves evaluated and were found to be 329, 70, 235, 298, 107 and 31.2 g $kg^{-1}$ of DM, respectively. Likewise, the contents of DM, Ash, CP, NDF and EE of alfalfa hay were evaluated and found to be 900, 100, 170, 425 and 16 g $kg^{-1}$ of DM, respectively.

**Table 1.** Ingredients and chemical composition of experimental diets used for the *in vitro* gas production parameter, digestibility and the *in situ* DM degradability evaluation.

| Ingredients (%) | Experimental diets[3] | | | |
|---|---|---|---|---|
| | Control | 25% | 50% | 100% |
| Chopped alfalfa | 18 | 9 | 4.5 | - |
| Wheat straw | 17 | 17 | 17 | 17 |
| Wheat bran | 12 | 12 | 12 | 12 |
| Corn grain | 25 | 25 | 25 | 25 |
| Barley grain | 13 | 13 | 13 | 13 |
| Corn silage | 14 | 14 | 14 | 14 |
| Salt | 0.5 | 0.5 | 0.5 | 0.5 |
| *L. leucocephala* leaves | - | 4.5 | 9 | 18 |
| Mineral and vitamin supplement[1] | 0.5 | 0.5 | 0.5 | 0.5 |
| Chemical composition (%)[2] | | | | |
| ME (Mcal $kg^{-1}$) | 2.30 | 2.32 | 2.32 | 2.34 |
| DM | 79.6 | 72.9 | 70.4 | 69.3 |
| CP | 10.9 | 10.4 | 10.7 | 12.0 |
| EE | 2.39 | 2.73 | 3.14 | 4.03 |
| NDF | 37.0 | 34.5 | 33.9 | 34.7 |
| ADF | 19.1 | 19.1 | 19.0 | 18.8 |
| ADL | 4.07 | 4.41 | 4.45 | 4.83 |
| Ash | 5.68 | 5.09 | 4.96 | 5.32 |

[1]Provided the following (per kg of DM diet): Mn: 2,200 mg; Ca; 195 g; P: 80 g; Mg: 21,000 mg; Fe: 3,000 mg; Zn: 300 mg; Cu: 300 mg; I: 12 mg; Se: 1.1 mg; Co: 100 mg; 6,000 IU Vitamin A; 2,000 IU Vitamin D; 200 mg Vitamin E. [2]DM; Dry matter, ME; Metabolizable energy, CP; Crude protein, EE; Ether extract, NDF; Neutral detergent fiber, ADF; Acid detergent fiber, ADL; Acid detergent lignin. [3]% of *L. leucocephala* leaves replacing dietary alfalfa hay.

## Chemical analysis

Before the chemical analysis commence, feed samples were oven-dried at 55°C for 48 h to evaluate DM content and then were passed through a 1 mm sieve (Wiley mill, Swedesboro, USA). Following the AOAC International procedure, samples were analyzed for CP (N × 6.25; No. 988.05), EE (No. 920.39), Ash (No. 924.05) and acid detergent fiber (ADF) (No. 973.18; Association of Official Analytical Chemists [AOAC], 1998). Furthermore, NDF was analyzed according to Van Soest, Robertson, and Lewis (1991). Acid detergent lignin (ADL) was determined by solubilization of cellulose with a sulfuric acid (Robertson & Van Soest, 1980). Total tannins (TT) of *L. leucocephala* leaves were measured according to Makkar (2000).

## Biogas production parameters and digestibility

To investigate the *in vitro* GP parameters and the diets digestibility, rumen fluid was collected from four fistulated male buffalos before morning feeding. The animal was offered a diet composed of 60 forage and 40% concentrate a month prior to beginning of the experiment. A pump was used to sample the rumen liquor, which was immediately poured into a thermo-container pre-warmed at 39°C and pre-flushed with $CO_2$. Thereafter it was transferred to the ruminant nutrition laboratory located at the Agricultural Sciences and Natural Resources University of Khuzestan pH of the rumen liquor was immediately measured by portable pH meter (Metrohm model, Swiss). The rumen liquor was filtered by using 4-layer cheesecloth and poured into the plastic tube which was placed in a warm water-bath at 39°C.

The artificial saliva was prepared according to Menke and Steingass (1988) (includes distilled water, buffering solution, resazurin solution, macro mineral solution). Feed samples of 200 mg of each experimental diets with particle size 1 mm were placed in 100 mL glass vial at 39°C (6 replicates for each treatment). Then, 30 mL of mixed ruminal fluid and artificial saliva were added to each vial at a ratio of 1:2 and incubate at 39°C. GP was recorded at 2, 4, 6, 8, 12, 24, 48, 72 and 96 hour after incubation. GP data was fitted to the modified model described by Ørskov and McDonald (1979) as follows Equation 1:

$$y = a + b\,(1 - e^{-ct}) \quad (1)$$

where:
y is the volume of GP at the time t in mL,
b is GP from the fermentable fraction (mL 200 $mg^{-1}$ of DM),
c is the GP rate constant (mL $hour^{-1}$) and t is the incubation time (hour).

The organic matter digestibility (OMD) and metabolizable energy (ME) were estimated according to the following Equation 2 and 3:

$$\text{OMD (g kg}^{-1}\text{ of DM)} = [148.8 + (8.89 \times GP_{24}) + (4.5 \times CP) + (0.651 \times \text{ash})] \text{ (Menke et al., 1979)} \quad (2)$$

$$\text{ME (MJ kg}^{-1}\text{ of DM)} = [2.2 + (0.136 \times GP_{24}) + (0.057 \times CP)] \text{ (Menke et al., 1979)} \quad (3)$$

The 96 hour apparently degraded substrate (ADS) content (mg $g^{-1}$ of DM) was calculated as the difference between DM content of substrate and its undegradable DM (Salem et al., 2013). The partitioning factor (PF), microbial protein (MCP) and short chain fatty acids (SCFA) were estimated according Equation 4 to 6.

$$\text{PF (mg mL}^{-1}) = \frac{\text{ADS (mg)}}{\text{GP96 (mL)}} \text{ (Blümmel, Steingaß, \& Becker, 1997)} \quad (4)$$

$$\text{MCP (mg g}^{-1}\text{ of DM)} = \text{ADS} - (\text{mL } GP_{24} \times 2.2 \text{ mg mL}^{-1}) \text{ (Blümmel et al.,1997)} \quad (5)$$

where:

The 2.2 mg per mL is a stoichiometric factor that expresses milligrams of carbon, hydrogen, and oxygen required for the production of SCFA gas complex associated with production of 1 mL of gas (Blümmel et al., 1997).

$$\text{SCFA (mmol 200 mg}^{-1}\text{ of DM)} = (0.0222 \times GP_{24}) - 0.00425 \text{ (Getachew, Makkar, \& Becker, 2002)} \quad (6)$$

where:

$GP_{96}$ is the volume of GP at the 96 hour incubation, $GP_{24}$ is the volume of GP at the 24 hour incubation and CP is crude protein (g $kg^{-1}$ of DM).

The *in vitro* digestibility of DM (IVDMD) of experimental diets was determined using Tilley and Terry (1963) method. Therefore, rumen liquor was collected and mixed with McDougall buffer solution (includes [g $L^{-1}$] 9.8 Sodium Bicarbonate, 2.44 Sodium Phosphate Dibasic, 0.57 Potassium Chloride, 0.47 Sodium Chloride,

0.12 Magnesium Sulfate and 0.16 Calcium Chloride) in a ratio of 1:4. After flushing with $CO_2$, tubes were incubated at 39ºC for 48 hour incubation. Thereafter, 6 mL of 20% HCl solution and 5 mL pepsin solution were added and then incubated for 48h simulating post-ruminal degradation. The residual substrates of each tube were filtered and used to determine the digestibility of DM.

### *In situ* degradability of DM

To investigate the *in situ* degradability of DM, we used the nylon bag method according to (AFRC, 1993). A bags representing the experimental treatments were suspended in the rumen of four rumen-fistulated buffalos (their average body weight was 450 kg and of 3 years old). Animals were fed at 0700 and 1900 hour with a diet composed of 40 concentrate and 60% forage. The concentrate was made of corn and barley grains, wheat bran, vitamin and mineral premix. Whereas the offered forage were alfalfa hay and wheat straw and corn silage. The adaptation period lasted for 14 days. The experimental diets (Table 1) were milled and passed from a 2 mm screen. Then 5 mg of each diet were poured into Dacron bags (10 x 20 cm diameter, and 53 ± 10 µm pore size; R1020, Ankom Technology, Macedon, NY, USA). Different treatment bags were placed in the rumen simultaneously and in 3 replicates each time (96 bags). Nylon bag were incubated for 2, 4, 8, 12, 24, 48, 72 and 96 hour. After incubation, bags were withdrawn from the rumen and washed with cold water. The value of degradability at time zero was obtained by washing three bags per sample with cold water. Difference between primary weight with the weight after incubation, the disappearance rate of DM, was calculated at each of the incubation times. The degradability data obtained for each treatment was fitted to the modified model described by Ørskov and McDonald (1979) as $P = A + B(1-e^{-Ct})$ where P is the degradation rate at time t, A is the rapidly soluble fraction, B is the insoluble but potentially degradable fraction; C is the rate of degradation of fraction B; and t is the incubation time. Moreover, the effective degradability (ED) was calculated according to the Equation 7:

$$ED = A + (B \times \frac{C}{C+K}) \text{ (Ørskov \& McDonald, 1979)} \quad (7)$$

where:
K is the estimated rate of outflow from the rumen. The outflow from the rumen rate was considered 0.02, 0.05 and 0.08 hour.

### Statistical analysis

Data on *in vitro* GP, *in vitro* digestibility and *in situ* degradability experiment were analyzed using a mixed model (Mixed procedures) of Statistical Analysis System (SAS, 2008) as follows Equation 8:

$$Y_{ij} = \mu + D_i + e_{ij} \quad (8)$$

where:
$Y_{ij}$ is the $K^{th}$ observation of the dependent (response) variable for the specific $ij^{th}$ animal, $\mu$ is the overall mean, Di is the effect of $i^{th}$ treatment, $e_{ij}$ is the random residual error. Means were compared by the Duncan multiple comparison tests at $p < 0.05$.

## Results and discussion

The effect of replacing dietary alfalfa hay with varying levels of *L. leucocephala* leaves on the *in vitro* rumen gas kinetics (b [potential of GP] and c [GP rate constant]) and GP are shown in Table 2.

The values of b and c decreased significantly decreased by increasing the level of *L. leucocephala* leaves ($p < 0.05$). Despite of nutrients contents of *L. leucocephala* leaves (Norton & Poppi, 1995), it's potential of GP (b) was less than the control treatment where Alfalfa hay was a major roughage. The GP volume between 2 and 12 hour differed significantly between treatments. However, the cumulative GP after 12 hour incubation did not differ significantly ($p > 0.05$) between experimental treatments, indicating a decline in tannins effect in a later incubation period. In our study, we evaluated tannins content of *L. leucocephala* leaves and was 31.2 g $kg^{-1}$ of DM. It could be that the presence of anti-nutritional compounds in the *L. leucocephala* leaves reduced the potential for GP by increasing its dietary inclusion level. Tannins has been reported to reduce the fermentation, digestibility of nutrients and methane production (Beauchemin, McGinn, Martinez, & McAllister, 2007) by reducing of potential of microorganisms to bind to feed particles (McAllister, Bae, Jones, & Cheng, 1994). Furthermore, tannins inhibit the growth of microbial enzymes (McSweeney, Palmer, McNeill,

& Krause, 2001). The negative correlation between tannins content and the GP volume found in our study agree with the findings of Khazaal, Boza, and Ørskov (1994) and with Chaji, Direkvandi, and Salem (2020) who found that the presence of tannin reduced GP due to the disruption effect on rumen microorganisms.

Table 3 shows the effect of replacing dietary alfalfa hay with different levels of *L. leucocephala* leaves on *in vitro* GP parameter and *in vitro* digestibility of the evaluated diets.

**Table 2.** Effect of alfalfa hay replacing with different levels of *L. leucocephala* leaves on *in vitro* rumen gas kinetics and cumulative gas production (mL 200 $mg^{-1}$ of DM).

| Parameter[1] | Control | % of *L. leucocephala* | | | SEM[2] | p-Value |
|---|---|---|---|---|---|---|
| | | 25 | 50 | 100 | | |
| b | 56.1[a] | 54.4[b] | 52.5[c] | 51.5[c] | 0.53 | 0.001 |
| c | 0.032[a] | 0.028[b] | 0.022[d] | 0.025[c] | 0.001 | 0.001 |
| Incubation time (hours) | | | | | | |
| 2 | 0.63[b] | 1.26[a] | 0.43[b] | 0.63[b] | 0.20 | 0.001 |
| 4 | 3.18[b] | 4.22[a] | 1.91[c] | 2.96[b] | 0.28 | 0.002 |
| 6 | 5.91[a] | 5.86[a] | 3.35[b] | 5.12[a] | 0.51 | 0.005 |
| 8 | 13.7[a] | 9.83[b] | 7.65[c] | 11.7[ab] | 0.72 | 0.002 |
| 12 | 19.0[a] | 13.8[bc] | 11.6[c] | 15.0[b] | 1.20 | 0.014 |
| 24 | 32.8 | 29.5 | 29.5 | 30.4 | 1.85 | 0.563 |
| 48 | 45.3 | 42.5 | 44.3 | 44.6 | 1.43 | 0.523 |
| 72 | 50.6 | 47.9 | 50.6 | 50.6 | 1.33 | 0.421 |
| 96 | 52.1 | 48.5 | 52.1 | 51.7 | 1.33 | 0.241 |

[1]b: Gas production from the fermentable fraction (mL 200 $mg^{-1}$ of DM), c: Gas production rate constant (mL $hou^{-1}$). [2]SEM: standard error of means. [a-d]Means in the same row with different superscript letters differ significantly ($p < 0.05$).

**Table 3.** Effect of alfalfa hay replacing with replacing alfalfa hay with different levels of *L. leucocephala* leaves on *in vitro* gas production parameter and *in vitro* digestibility.

| Parameter[1] | Control | % of *L. leucocephala* | | | SEM[2] | p-Value |
|---|---|---|---|---|---|---|
| | | 25 | 50 | 100 | | |
| 24 hour incubation | | | | | | |
| OMD (mg $g^{-1}$ of DM incubated) | 449[a] | 419[b] | 420[b] | 379[c] | 14.5 | 0.01 |
| ME (MJ $kg^{-1}$ of DM) | 7.36[a] | 6.91[b] | 6.91[b] | 6.00[c] | 0.22 | 0.01 |
| MCP (mg $g^{-1}$ of DM) | 570[b] | 589[a] | 599[a] | 531[c] | 8.43 | 0.01 |
| SCFA (mmoL 200 $mg^{-1}$ of DM) | 0.725[a] | 0.650[b] | 0.650[b] | 0.550[c] | 0.02 | 0.01 |
| 96 hour incubation | | | | | | |
| ADS (mg $g^{-1}$ of DM) | 640[b] | 654[ab] | 664[a] | 586[b] | 9.10 | 0.01 |
| PF | 12.3 | 13.5 | 12.8 | 13.0 | 0.55 | 0.20 |
| *In vitro* DM digestibility (g $kg^{-1}$ of DM) | | | | | | |
| IVDMD | 730[a] | 741[a] | 764[a] | 704[b] | 11.4 | 0.01 |

[1]OMD (organic matter digestibility), ME (metabolizable energy), MCP (microbial CP), ADS (apparently degraded substrate), PF (partitioning factor), SCFA (short chain fatty acids), IVDMD: *In vitro* digestibility of dry matter. [2]SEM: standard error of means. [a-c]Means in the same row with different superscript letters are different ($p < 0.05$).

Replacing alfalfa hay with varying amounts of *L. leucocephala* affected the dietary contents of OMD, ME, SCFA, MCP, ADS and the IVDMD. The highest OMD, ME and SCFA contents were observed in the control diet ($p < 0.05$), than those diets containing *L. leucocephala* leaves. The low OMD digestibility of *L. leucocephala* leaves could be in part explained by the negative effect of tannins on fermentation and digestibility. Tannins can be complexed with other nutrients during the *in vitro* incubation leading to a reduced OMD (Sallam et al., 2010). Whereas the MCP after 24 hour incubation and the ADS content after 96 hour incubation, increased significantly when *L. leucocephala* leaves replaced alfalfa hay by 50% ($p < 0.05$). It was reported that the addition of saponin sources to alfalfa hay, increased the *in vitro* MCP linearly, due to its negative effect on rumen protozoa. Despite we did not evaluate the rumen protaoza in the current study, it was reported that when rumen protozoa decreases, the amount of bacterial predation decreases as well and lead to an increase in the microbial N flow to the duodenum. Furthermore, Barros-Rodríguez et al. (2015) reported that rumen protozoa decreased when sheep were fed with 20 and 40 percent *L. leucocephala*. Therefore, a decline in rumen protozoa in the *L. leucocephala* containing diets in the current study could be speculated. The PF shows the rate of substrate digestion to the volume of GP, volatile fatty acids (VFAs) and microbial biomass. There is a negative correlation between GP and PF ratio, as when the GP volume increases the amount of PF decreases (Blümmel et al., 1997). The PF ratio in our study agree with those reported by Soltan, Morsy, Sallam, Louvandini, and Abdalla (2012) who compared between PF value of *L. leucocephala* treatment and Tifton hay. The later reported that the increase in PF rate in *L. leucocephala* was associated with the reduction of methane

GP. However, in our experiment, although PF numerically increased by including *L. leucocephala* in the diets, there was no significant difference between treatments ($p > 0.05$). This could be explained by the presence of tannins that leads to the increase in PF rate. Hence, the higher proportion of digestible nutrients was used to produce MCP compared the synthesis of SCFA as reported by Angaji, Souri, and Moeini (2011) which could explain the increase in MCP and SCFA in treatments that contain 50% *L. leucocephala* leaves.

There was no significant difference between IVDMD of 25 and 50% *L. leucocephala* leaves compared to the control diet. Paengkoum (2010) found that using *L. leucocephala* leaves in the diet decreased the digestibility of DM which agrees with our results at a complete substitution of alfalfa hay by *L. leucocephala*. Nasimi, Chaji, Mohammadabadi, and Bojarpour (2016) explained the decline in nutrient digestibility due to the greater content of lignin in *L. leucocephala* leaves compared to alfalfa hay (11 vs. 9%) and due to the anti-nutritional factors that present in *L. leucocephala* leaves. The level of the IVDMD correlate negatively with the dietary concentration of the anti-nutritional agents which could explain in part the decline in *L. leucocephala* DM digestibility at full substitution. *In situ* DM degradability results are presented in Table 4.

Fraction A (the soluble and very rapidly degradable fraction) was not affected by the different *L. leucocephala* dietary levels ($p > 0.05$). Fraction B (the insoluble but potentially degradable fraction) and the potential of degradability (A+B) were significantly increased for treatment that contained 50% *L. leucocephala* leaves ($p < 0.05$). However, the fraction C increased significantly for treatments contains 25% *L. leucocephala* leaves ($p < 0.05$). The effective degradability $ED_2$ ($k = 0.02$), $ED_5$ ($k = 0.05$) and $ED_8$ ($k = 0.08$) increased significantly for treatment that contained 25% *L. leucocephala* leaves ($p < 0.05$).

Our results do not agree with Mondal, Walli, and Patra (2008) who found different *in situ* DM degradability values since they used *L. leucocephala* leaves as the only dietary component to determine the *in situ* DM degradability. The variations in the *in situ* DM degradability of B, A+B and ED could be explained by the nutrients availability of carbohydrates and protein in *L. leucocephala* pod compared to alfalfa hay (Shahriari, Mohammadabadi, Vakili, Chaji, & Sari, 2017).

In our study, $ED_2$, $ED_5$ and $ED_8$ decreased when *L. leucocephala* leaves replaced alfalfa hay at 50 and 100%. This can be due to the presence of anti-nutritional factors such as saponin and tannin in *L. leucocephala* leaves. Lu and Jorgensen (1987) reported that the presence of saponin in *L. leucocephala* leaves reduced the digestibility of *L. leucocephala* leaves. The presence of saponins in *L. leucocephala* reduce the effect of rumen fibrolytic enzyme thereby it disrupts the fiber digestion in the rumen. This was observed in our study as the effective degradability at rates of 0.02, 0.05 and 0.08 were significantly greater in alfalfa hay based diet at and at 25% *L. leucocephala* leaves substitution rate compared to higher dietary substitution rates of 50 and 100%. A high concentration of tannins was reported to reduce the digestibility of proteins and carbohydrates and has a negative effect on animal's performance (Reed, Soller, & Woodward, 1990).

**Table 4.** *In situ* DM degradability of diets with replacing alfalfa hay with different levels of *L. leucocephala* leaves.

| Parameter[1] | Control | % of *L. leucocephala* | | | SEM[2] | p-Value |
|---|---|---|---|---|---|---|
| | | 25 | 50 | 100 | | |
| A (g $kg^{-1}$ of DM) | 234 | 234 | 236 | 234 | 1.41 | 0.761 |
| B (g $kg^{-1}$ of DM) | $555^{c}$ | $653^{b}$ | $700^{a}$ | $663^{b}$ | 7.00 | 0.001 |
| C ($hour^{-1}$) | $0.081^{b}$ | $0.090^{a}$ | $0.013^{d}$ | $0.020^{c}$ | 0.002 | 0.001 |
| A+B (g $kg^{-1}$ of DM) | $790^{c}$ | $887^{b}$ | $935^{a}$ | $897^{b}$ | 17.8 | 0.001 |
| ED (g $kg^{-1}$ of DM) | | | | | | |
| $k_{0.02}$ $hour^{-1}$ | $679^{a}$ | $764^{a}$ | $509^{c}$ | $552^{c}$ | 34.6 | 0.001 |
| $k_{0.05}$ $hour^{-1}$ | $578^{a}$ | $648^{a}$ | $378^{b}$ | $412^{b}$ | 42.8 | 0.001 |
| $k_{0.08}$ $hour^{-1}$ | $514^{a}$ | $573^{a}$ | $332^{b}$ | $357^{b}$ | 43.8 | 0.001 |

[1]A: soluble and very rapidly degradable fraction (% of DM), B: insoluble but potentially degradable fraction (% of DM), C: fractional degradation rate of B ($hour^{-1}$), A+B: potential of degradability, ED: effective degradability calculated for an outflow rates of 0.02, 0.05 and 0.08 $hour^{-1}$, respectively. [2]SEM: standard error of means. [a-d]Means in the same row with different superscript letters are different ($p < 0.05$).

## Conclusion

Our results showed that substituting dietary alfalfa hay with *L. leucocephala* leaves at levels of 25 to 50% had no negative effects on the GP parameter and *in situ* digestibility of DM in buffalo. A full substitution of alfalfa hay by *L. leucocephala* reduced the *in vitro* and *in situ* digestibility parameters which suggest further treatments to reduce their negative effect on animal performance before feeding. Such a treatment needs to be evaluated from economic perspective in comparison with a good quality feeds such alfalfa hay. Overall, *L. leucocephala* could be an important alternative forage source for ruminants in arid areas.

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