



# **Agronomic Yield, Digestibility, and Protein Content of Nine Alfalfa (*Medicago sativa* L.) Varieties in Central Valley Oaxaca Mexico**

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## **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

The aim of the present study was to evaluate the yield, digestibility, and protein content of nine varieties of alfalfa (*Medicago sativa* L.). The varieties of alfalfa were compared during a complete production cycle. A completely randomized design was used taking into account the season of the year as a block and the varieties as treatment, a Tukey test was used to evaluate differences between treatments. The highest dry matter (DM) production was in the summer, with Atlixco variety being the highest yield (1953.3 kg DM cut<sup>-1</sup>), the ratio leaf:stalk (L:S) was highest during the winter, with no differences ( $p>0.05$ ) between varieties. The varieties with highest protein content (PC) was Caliverde and Valenciana (22.9 % PC) during the winter ( $p<0.05$ ). The highest digestibility was in the summer and there were no differences between varieties. The higher amount of digestible dry matter (DDM) and PC were produced by Valenciana (1586.7 kg DDM cut<sup>-1</sup> and 446.7 kg PC cut<sup>-1</sup>), UC-Cibola (1557.0 kg DDM cut<sup>-1</sup> and 432.3 kg PC cut<sup>-1</sup>) and Atlixco (1549 kg DDM cut<sup>-1</sup> and 441.1 kg PC cut<sup>-1</sup>). The highest dry matter production was during the summer and spring, the three most productive varieties were Valenciana, Atlixco and UC-Cibola, and therefore these are the most suitable varieties for the best quality forage production in the region of study.

*Keywords: Alfalfa; yield; digestibility; protein content.*

## 1. INTRODUCTION

Alfalfa (*Medicago sativa* L.) is the most important perennial leguminous fodder in the world, its cultivated in more than 80 countries, approximately 30% of the occupied surface by leguminous it is dedicated to alfalfa [1], it is one of the most important feed for ruminants, for its nutritional quality it is one of the main ingredients for dairy cattle [2,3]. Alfalfa it's known as the "queen of forages" because of its high dry matter yield, digestibility, high protein, vitamin and mineral content [4]. It's used as a soil remedy, due to its capacity to modulate de nitrogen and carbon cycle, helping to reduce the greenhouse gas emissions, it has a deep root system that allows it to grow well in marginal conditions without fertilization including semi-arid lands and with limited irrigation [5]. Its agronomic performance has been evaluated in many studies to ensure high productivity considering cut interval and the season of the year, also the speed of resprout between cuts and the remain leaf area is fundamental to understand the effect of frequency and intensity of the forage yield [6]. On the other hand, Montes [7] and Rojas [8] report a high growth rate in alfalfa in spring and summer seasons, and less growth in the fall and winter in cold weather in the Mexico Valley. However, Villegas [9] obtained a high growth rate of two varieties of alfalfa in in spring, followed by the winter, summer and the lowest growth rate in the fall in the Oaxaca Valley. Rivas [10] and Zaragoza [11], without considering interval yields, obtained higher yields for alfalfa in spring-summer, than in the winter. Villegas [9] obtained the highest yield with two varieties in spring,

winter and summer and the lowest production in the fall. All of the carried out studies have created a great technical knowledge about alfalfa biology, which in turn lead in the development of new varieties that can grow in different environmental conditions and management [12-14]. In Mexico many studies about productive variables of alfalfa have been performed, however, there is not a definition of which varieties would be more appropriated for each region. For all the above mentioned, the aim of the present study was to evaluate yield, digestibility and protein content of nine alfalfa varieties, in the Oaxaca central Valleys, Mexico.

## 2. MATERIALS AND METHODS

### 2.1 Localitation

The experiment was carried out during the 2018 production cycle in the fields of the unit dairy production facility of Nazareno, Etila Oaxaca, at 17°13' N and 96°48'W at 1641 meters above sea level inside the region named Oaxaca Central Valley, Mexico, with a semi warm weather with isothermal fresh summer (A)C(W'o)(W)b(i'g), with an average annual temperature 19.2°C and average annual rainfall 667 mm [15]. The predominant soil type in the region is cambisol calcium [16].

### 2.2 Plot Management

Four 5x5 m sampling plots were randomly established; the prairie was in the second-year

yield, interval cut varied according to the season of the year and was established by the following way: 35 days in spring, 28 days in the summer, 35 days in the fall and 42 days in the winter, a total of 19 irrigation water periods were performed on a two-week interval between each irrigation. The varieties used were Atlixco, Caliverde, El camino, UC-Cibola, Moapa, NK-819, Oaxaca, Valenciana and Velluda Peruana.

## 2.3 Measured Variables

### 2.3.1 Dry matter yield

Dry matter (DM) yield per cut was evaluated using a sample unit a square of 0.25 m<sup>2</sup> in each alfalfa plot, taking two random samples per repetition. The fodder in each square was harvest one day before the cut, leaving a 5 cm remain height, samples were weighed in fresh, then they were washed and dried in an air force stove at a 55 °C temperature during 72 h once the fodder sample was dried its dry weight was recorded, in order to determine the yield by surface unit (kg DM ha<sup>-1</sup>).

### 2.3.2. Ratio leaf: stalk (L:S)

From the fresh fodder samples, 100 g were separated, from these leaf and stalk were separated to calculate the leaf:stalk ratio (LSR), and it was calculated with the following formula:

$$L:S= L/S$$

where:

L= dry leaf weight (g); S= dry stalk weight (g).

### 2.3.3 Crude protein

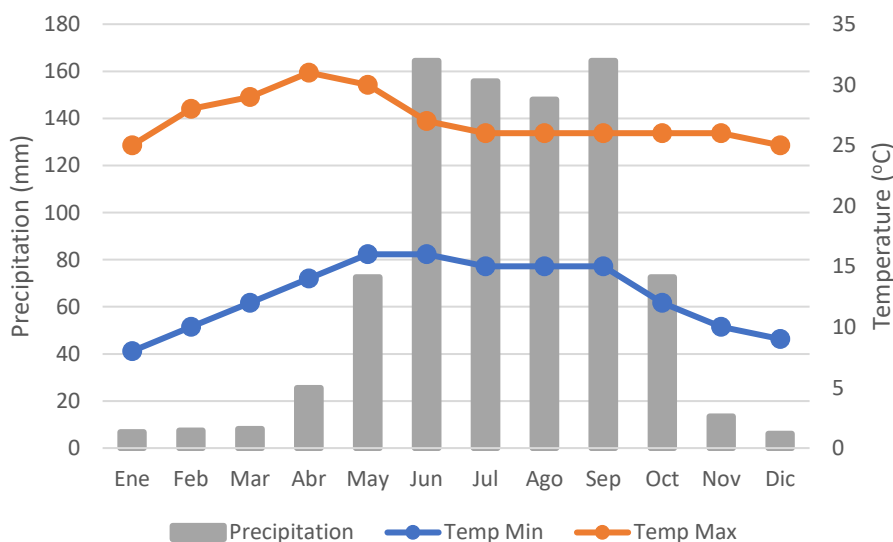
For the protein determination a Kjeldahl method [17], was used, using a 2 g ground sample dry matter, they were digested and then distilled in a micro Kjeldahl equipment; the distilled was titrated with boric acid, until it turned brilliant red, the milliliters spent were multiplied by 6.25 to obtain the protein percentage of each sample. In order to obtain the amount of protein produced, the protein percentage was multiplied by the DM kg of each sample.

### 2.3.4 Digestibility

For in situ digestion of dry matter (DISMS) an interval of 72 h was established [18]. With the data obtained of the digestibility percentage, data was multiplied by the DM kg of each sample of each variety, to obtain the amount of digestible dry matter digestibility (DISMS).

## 2.4 Weather Data

Data from the meteorological station were used, Oaxaca International Airport, this station is considered reliable according to the International Standard Atmosphere [19].



**Fig. 1. Monthly accumulated precipitation and minimum and maximum monthly average temperature in the Central Valleys of Oaxaca, Mexico**

## 2.5 Statistical Analysis

The variance analysis to analyze the effect of the alfalfa varieties used in this experiment, was performed by a mixed model procedure analysis of variance [20], with a complete random block design with four repetitions. Tukey test was used to compare means of treatments ( $\alpha = 0.05$ ) according to Steel and Torrie [21].

## 3. RESULTS AND DISCUSSION

### 3.1 Dry Matter Production

Average yield per cut (kg DM ha<sup>-1</sup>) of the nine alfalfa (*Medicago sativa*) varieties in the four seasons of the year are shown in Table 1, in average they produced 2081.1 kg per cut; Atlixco, El camino, UC-Cibola, Oaxaca, and Valenciana varieties, had a similar yield ( $p > 0.05$ ) outstanding Valenciana variety, that was statistically different than Caliverde, Moapa, Velluda ( $p < 0.05$ ), NK-819 variety had a lower production ( $p < 0.05$ ); the dry matter yield per cut per season of the year had the following order: summer > spring > fall > winter.

The average production per cut was similar to the one reported by Rivas-Jacobo [10], however, the varieties Oaxaca, Moapa and Valenciana in our study had less production per cut per season than the one reported by these authors.

In a study made in the region of Valles Centrales of Oaxaca with the varieties: Moapa, Oaxaca and Valenciana by Villegas-Aparicio [22], in general the average yield was similar to our results, but it was higher in spring and summer, and during the fall and winter decreased markedly. By year season winter production was lower ( $p < 0.05$ ) and in the other seasons was similar ( $p > 0.05$ ).

The highest production per cut was achieved in the summer, where varieties Atlixco and Velluda were different to El Camino and Moapa with less yield per cut ( $p < 0.05$ ). During the winter all varieties had less production due to the decrease of temperature and light hours. The highest growth rate of alfalfa was in spring and summer with presence of optimum temperatures according with data reported by Rojas [23].

Rojas [8] obtained an average annual yield of 1505 kg of DM ha<sup>-1</sup> cut<sup>-1</sup> in five varieties of alfalfa in Mexico Valley, which is under the data found in our study, as regards of Atlixco variety which was also evaluated by our team, it also had a

better yield per cut, however, during the summer they observed a higher yield of the mentioned variety 2072 kg DM ha<sup>-1</sup> cut<sup>-1</sup>, the higher results found in our study maybe were due to more constant temperatures during the cycle of evaluation.

### 3.2 Leaf:Stalk Ratio (L:S)

In the leaf:stalk ratio (L:S) there were only differences by season in the UC-Cibola and NK-819 varieties (Table 2), the variety UC-Cibola in the fall had a lower ratio L:S ( $p < 0.05$ ) and in the winter the variety NK-819 has a higher ratio L:S ( $p < 0.05$ ), in the remain varieties de L:S was similar ( $p > 0.05$ ), during all the seasons of the year, in the winter the was the highest ratio L:S and in the summer the lowest ( $p < 0.05$ ). During the fall there were only differences between varieties, where Atlixco had the highest ratio L:S, these results are in agree with those reported by Alvarez-Vázquez [24], where in the fall the Atlixco, Oaxaca and Valenciana varieties had the highest ratio L:S.

The average L:S was different in the seasons of the year, with the higher values in the winter and fall ( $p < 0.05$ ), which are in agree with the one reported by Rojas García [25], in the summer the ratio is lower. In general, the ratio L:S is similar to the one reported by Urbano [26] in a study in the high zone of Venezuela. The L:S in our study was lower to the one reported in the fall and winter 1.46 and 1.52, but was higher to what they reported in spring and summer, 0.94 y 0.92, in general our results were more constant, during the four seasons of the year, in average the L:S was 1.27. The L:S in forages it's an indirect measure of quality, if the value is higher than one, it indicates better forage quality than the values lower than one [9,27,28] in our study all the varieties scored values higher than one, which indicates forage of good quality, which is related to a good digestibility and protein content.

The lower ratio L:S that was in the summer and spring it's because in these seasons the stalk growth is faster than the leaf production, decreasing de L:S which is in agreement with the one reported by Rojas [8].

### 3.3 Protein Content (PC)

The protein content is shown in Table 3, there were no differences between varieties ( $p > 0.05$ ). Between seasons, in the winter there was the higher protein content and, in the spring, the

lowest content ( $p < 0.05$ ); in the summer and the fall there were no ( $p > 0.05$ ) differences. The Moapa, Caliverde and Valenciana varieties had a higher protein content trend in the winter and the Atlitxco variety had a lower protein content in the

spring. In the majority of the leguminous the green parts of alfalfa are rich in protein, and its ability to attach atmospheric nitrogen makes alfalfa an important crop for forage production and soil conservation [29].

**Table 1. Average dry matter yield of nine alfalfa varieties per season (kg DM ha<sup>-1</sup>cut<sup>-1</sup>) per alfalfa cut in the Central Valleys of Oaxaca, Mexico**

Varieties	Winter	Spring	Summer	Fall	Mean
Atlixco	1501.1 <sup>cA</sup>	1812.4 <sup>bA</sup>	1953.3 <sup>aA</sup>	1745.4 <sup>bBCD</sup>	1753.0 <sup>AB</sup>
Caliverde	1554.4 <sup>bA</sup>	1655.2 <sup>abBCD</sup>	1758.8 <sup>aBC</sup>	1527.3 <sup>bE</sup>	1623.9 <sup>BC</sup>
El Camino	1199.4 <sup>cB</sup>	1852.8 <sup>aA</sup>	1635.5 <sup>bC</sup>	1907.3 <sup>aA</sup>	1648.7 <sup>ABC</sup>
UC-Cibola	1495.9 <sup>bA</sup>	1764.2 <sup>aAB</sup>	1852.2 <sup>aAB</sup>	1797.2 <sup>aABC</sup>	1727.4 <sup>AB</sup>
Moapa	1528.5 <sup>bA</sup>	1542.2 <sup>abD</sup>	1710.2 <sup>aC</sup>	1692.7 <sup>abDC</sup>	1618.4 <sup>CB</sup>
NK-819	1375.1 <sup>bAB</sup>	1618.7 <sup>aCD</sup>	1764.4 <sup>aBC</sup>	1318.5 <sup>bF</sup>	1519.2 <sup>C</sup>
Oaxaca	1850.3 <sup>bAB</sup>	1727.1 <sup>aABC</sup>	1850.3 <sup>aAB</sup>	1732.2 <sup>aBCD</sup>	1699.4 <sup>AB</sup>
Valenciana	1569.9 <sup>bA</sup>	1834.9 <sup>aA</sup>	1886.6 <sup>aAB</sup>	1870.8 <sup>aAB</sup>	1790.5 <sup>A</sup>
Velluda	1347.3 <sup>cAB</sup>	1584.3 <sup>bD</sup>	1932.8 <sup>aA</sup>	1606.7 <sup>bEF</sup>	1617.8 <sup>BC</sup>
Promedio	1451.1 <sup>b</sup>	1710.2 <sup>a</sup>	1816.0 <sup>a</sup>	1688.7 <sup>a</sup>	1666.5

*abc*=Means with the different lowercase literal per row are statistically different ( $p < 0.05$ )  
*ABCD*=Means with the different capital letter per column are statistically different ( $p < 0.05$ )

**Table 2. Leaf:stem ratio (L:S) of nine alfalfa varieties by season in the Central Valleys of Oaxaca, Mexico**

Varieties	Winter	Spring	Summer	Fall	Mean
Atlixco	1.42	1.19	1.25	1.66 <sup>aA</sup>	1.38
Caliverde	1.37	1.30	1.11	1.44 <sup>AB</sup>	1.31
El Camino	1.39	1.31	1.09	1.15 <sup>CB</sup>	1.23
UC-Cibola	1.47 <sup>a</sup>	1.25 <sup>ab</sup>	1.15 <sup>ab</sup>	0.90 <sup>bC</sup>	1.19
Moapa	1.49	1.16	1.15	1.29 <sup>B</sup>	1.27
NK-819	1.37 <sup>a</sup>	1.23 <sup>ab</sup>	1.03 <sup>b</sup>	1.30 <sup>abB</sup>	1.23
Oaxaca	1.32	1.28	1.28	1.26 <sup>B</sup>	1.29
Valenciana	1.30	1.31	1.17	1.46 <sup>AB</sup>	1.31
Velluda	1.39	1.12	1.10	1.26 <sup>B</sup>	1.22
Means	1.39 <sup>a</sup>	1.24 <sup>bc</sup>	1.15 <sup>c</sup>	1.30 <sup>ab</sup>	1.27

*abc*=Means with the different lowercase literal per row are statistically different ( $p < 0.05$ )  
*ABCD*=Means with the different capital letter per column are statistically different ( $p < 0.05$ )

**Table 3. Protein content (%) of nine varieties of alfalfa in the Central Valleys of Oaxaca, Mexico**

Varieties	Winter	Spring	Summer	Fall	Mean
Atlixco	22.1 <sup>aAB</sup>	17.7 <sup>bA</sup>	20.0 <sup>abA</sup>	21.1 <sup>aA</sup>	20.2 <sup>A</sup>
Caliverde	22.9 <sup>aA</sup>	18.9 <sup>bA</sup>	20.8 <sup>abA</sup>	20.5 <sup>abA</sup>	20.8 <sup>A</sup>
El Camino	22.2 <sup>aAB</sup>	18.7 <sup>bA</sup>	21.5 <sup>aA</sup>	20.1 <sup>abA</sup>	20.6 <sup>A</sup>
UC-Cibola	22.2 <sup>aAB</sup>	19.2 <sup>bA</sup>	19.7 <sup>abA</sup>	19.3 <sup>abA</sup>	20.1 <sup>A</sup>
Moapa	23.0 <sup>aA</sup>	19.1 <sup>cA</sup>	20.4 <sup>bcA</sup>	21.2 <sup>bA</sup>	20.9 <sup>A</sup>
NK-819	21.2 <sup>aBC</sup>	18.0 <sup>bA</sup>	19.1 <sup>abA</sup>	19.9 <sup>abA</sup>	19.5 <sup>A</sup>
Oaxaca	21.7 <sup>aABC</sup>	18.8 <sup>bA</sup>	19.1 <sup>bA</sup>	20.0 <sup>bA</sup>	19.9 <sup>A</sup>
Valenciana	22.9 <sup>aA</sup>	18.3 <sup>bA</sup>	19.7 <sup>abA</sup>	19.4 <sup>abA</sup>	20.1 <sup>A</sup>
Velluda	20.4 <sup>aC</sup>	18.1 <sup>bA</sup>	19.8 <sup>abA</sup>	19.2 <sup>abA</sup>	19.4 <sup>A</sup>
Mean	22.1 <sup>a</sup>	18.5 <sup>c</sup>	20.0 <sup>b</sup>	20.1 <sup>b</sup>	20.2

*abc*=Means with the different lowercase literal per row are statistically different ( $p < 0.05$ )  
*ABCD*=Means with the different capital letter per column are statistically different ( $p < 0.05$ )

**Table 4. Dry Matter Digestibility (%) of nine alfalfa varieties in the Central Valleys of Oaxaca, Mexico**

Varieties	Winter	Spring	Summer	Fall	Mean
Atlixco	72.2 <sup>ab</sup> <sub>A</sub>	65.5 <sup>b</sup> <sub>A</sub>	75.6 <sup>a</sup> <sub>A</sub>	69.4 <sup>ab</sup> <sub>A</sub>	70.7 <sub>A</sub>
Caliverde	74.1 <sup>a</sup> <sub>A</sub>	68.2 <sup>b</sup> <sub>A</sub>	69.0 <sup>b</sup> <sub>A</sub>	73.7 <sup>a</sup> <sub>A</sub>	71.2 <sub>A</sub>
El Camino	73.7 <sup>ab</sup> <sub>A</sub>	68.8 <sup>b</sup> <sub>A</sub>	75.0 <sup>a</sup> <sub>A</sub>	71.6 <sup>ab</sup> <sub>A</sub>	72.3 <sub>A</sub>
UC-Cibola	71.1 <sup>ab</sup> <sub>A</sub>	69.5 <sup>b</sup> <sub>A</sub>	72.6 <sup>ab</sup> <sub>A</sub>	74.8 <sup>a</sup> <sub>A</sub>	72.1 <sub>A</sub>
Moapa	72.5 <sup>ab</sup> <sub>A</sub>	68.7 <sup>b</sup> <sub>A</sub>	75.4 <sup>a</sup> <sub>A</sub>	69.9 <sup>b</sup> <sub>A</sub>	71.6 <sub>A</sub>
NK-819	71.4 <sup>ab</sup> <sub>A</sub>	66.5 <sup>bc</sup> <sub>A</sub>	75.2 <sup>a</sup> <sub>A</sub>	65.5 <sup>c</sup> <sub>A</sub>	69.7 <sub>A</sub>
Oaxaca	75.3 <sup>a</sup> <sub>A</sub>	73.2 <sup>a</sup> <sub>A</sub>	75.2 <sup>a</sup> <sub>A</sub>	66.5 <sup>b</sup> <sub>A</sub>	72.5 <sub>A</sub>
Valenciana	74.5 <sup>a</sup> <sub>A</sub>	71.1 <sup>a</sup> <sub>A</sub>	72.9 <sup>a</sup> <sub>A</sub>	65.6 <sup>b</sup> <sub>A</sub>	71.1 <sub>A</sub>
Velluda	72.6 <sup>a</sup> <sub>A</sub>	67.9 <sup>b</sup> <sub>A</sub>	71.8 <sup>ab</sup> <sub>A</sub>	71.7 <sup>ab</sup> <sub>A</sub>	71.0 <sub>A</sub>
Mean	73.1 <sup>a</sup>	68.8 <sup>b</sup>	73.6 <sup>a</sup>	69.9 <sup>b</sup>	

*abc=Means with the different lowercase literal per row are statistically different ( $p<0.05$ )*

*ABCD=Means with the different capital letter per column are statistically different ( $p<0.05$ )*

The results in our study are similar to those obtained in a research study done in Zacatecas by Sánchez [30], who reported in average 20.1 % de PC, in the five varieties used, and below of the results obtained by Urbano [26] in Venezuela who reported a protein content of 22.7 % PC, Chocarro [31] 24 % PC in alfalfa prairies in the winter and [32] who report 25.5 % PC at 30 days cut, similar to the days of cut in our study, however, at 40 days they reported a lower content (18.4 % PC) and in our study during the winter the interval cut was 42 days the protein content was higher. The results found in our study were higher to the ones reported by Zhang [33] who evaluated twenty crops in China and their average results were 18.0 % PC.

Protein production in kg per ha was obtained from multiplying dry matter yield produced by cut by the protein content of each variety, the total production was 418.4 kg of protein per cut in all varieties, Valenciana variety was the one with more ( $p<0.05$ ) production 446.7 kg of protein per cut and the variety NK-819 was the one with less production 369.0 kg protein; by season of the year, the summer had the highest ( $p<0.05$ ) with 453.1 kg protein and in the spring the production was lower ( $p<0.05$ ) 396.4 kg protein. In the summer the Atlixco variety was the one with the highest yield 488.1 kg PC and the one with lower yield was NK-819 420.2 kg protein ( $p<0.05$ ). Alfalfa contributes to the agriculture and livestock sustainability since its used to improve vegetal coverage, and prevents prairie degradation [31].

### 3.4 Dry Matter Digestibility

In average dry matter digestibility (DMD) was similar ( $p>0.05$ ) in the nine varieties, by season of the year the winter and summer had the

highest ( $p<0.05$ ) digestibility. Atlixco, El camino, Moapa, NK-819 and Oaxaca varieties had the highest digestibility in the winter and summer (Table 4). This is because in the winter due to a low temperature the physiological maturity stops and the digestibility increases, in the summer the highest digestibility is due to the alfalfa grows faster and its harvested at a less age (four weeks) [34,35].

The Valenciana variety had the highest ( $p<0.05$ ) dry matter digestibility with 1586.7 kg DM/cut, higher than Caliverde, El Camino, Moapa and Velluda (1443.3, 1484.1, 1448.2 and 1435 kg respectively), the NK-819 variety had the lowest digestibility with 1327.1 kg. By season of the year the summer had the highest dry matter digestibility 1669.7 kg DM cut<sup>-1</sup> and lower in the winter 1324.6 kg DM cut<sup>-1</sup> ( $p<0.05$ ). Atlixco variety in the summer had the highest production per cut 1844.1 kg DM cut<sup>-1</sup> and the El Camino variety had the lowest production during the winter. These results can be due to the lower temperatures recorded in the winter that stop the physiological maturity of the forage species making them more appropriated for the digestion process [34,35].

In terms of production and quality of the different varieties; Valenciana had (1790.5 kg DM cut<sup>-1</sup>, 1586.7 kg DMD cut<sup>-1</sup> and 446.7 kg protein cut<sup>-1</sup>), UC-Cibola (1790.5 kg DM cut<sup>-1</sup>, 1557.0 kg DMD cut<sup>-1</sup> and 432.3 446.7 kg CP cut<sup>-1</sup>) and Atlixco (1753.0 kg DM cut<sup>-1</sup>, 1549 kg DMD cut<sup>-1</sup> and 441.1 kg CP cut<sup>-1</sup>). The average highest values per cut coincide with the highest temperatures and rainfalls of the year.

#### 4. CONCLUSION

The highest dry matter yield was during the spring and summer, the three most productive varieties were Valenciana, Atlixco and UC-Cibola; in the L:S ratio Atlixco was the best variety; Moapa had the highest crude protein content and the most digestible was the Oaxaca variety. In terms of dry matter digestible production Valenciana, Atlixco and UC-Cibola varieties were the highest, that is why these three varieties are the most recommended for the forage production in the study region as they have a lower environmental impact due to dry matter production and protein content.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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