Scientific Paper

Productive evaluation and chemical composition of six genotypes of *Medicago sativa* L. in the Andes of northern Peru

Magali Beatriz Cubas-Leiva[1] *https://orcid.org/0000-0001-8194-9346*, Luis Asunción Vallejos-Fernández[1] *https://orcid.org/0000-0002-9858-642X*, Roy Roger Florián-Lescano[1] *https://orcid.org/0000-0002-9681-9576*, William Leoncio Carrasco-Chilón[2] *https://orcid.org/0000-0003-4930-6548* and Wuesley Yusmein Alvarez-García[1, 2] *https://orcid.org/0000-0002-9655-3149*

[1]*Facultad de Ingeniería en Ciencias Pecuarias, Universidad Nacional de Cajamarca. Av. Atahualpa 1050. Cajamarca 06003, Perú.* [2]*Estación Experimental de Baños del Inca, Programa Nacional de Pastos y Forrajes, Dirección de Desarrollo Tecnológico Agrario, Instituto Nacional de Innovación Agraria (INIA), Jr. Wiracocha s/n, Baños del Inca, Cajamarca 06004, Perú. E-mail:* maga10243@gmail.com, lvallejos@unc.edu.pe, rflorian@unc.edu.pe, wcarrasco@inia.gob.pe, wuesley.alvarez@hotmail.com, walvarezg@unc.edu.pe

Abstract

Objective: To evaluate the yield, growth rate, plant height and crude protein, ash and neutral detergent fiber contents in six genotypes of *Medicago sativa* L. and on two high Andean plateaus of northern Peru.

Materials and Methods: A complete randomized block design was used. The *M. sativa* varieties (SW10, W450, HORTUS 401, W350, SW8210 and STAMINO 5) were considered the treatments, distributed in three blocks, repeated in the two localities, with a total of 36 experimental units. The chemical composition, green forage and dry matter yield, growth rate and plant height in the six genotypes and their interaction, were evaluated. The analysis of the productive indicators was done through variance analysis. In addition, Pearson's correlation tests were carried out to find relation between plant height and biomass yield.

Results: The altitudinal plateau I achieved 10 241,8 kg/ha/cut of green forage and 61 450,6 kg/ha/year, 1 739,9 kg/ha/cut and 10 498 kg/ha/year of dry matter, with the best production. The variety W-450 had higher average green forage yield (56 963 kg/ha/year) and dry matter (10 483,9 kg/ha/year). Plant height varied from 22,4 to 35,5 cm and crude protein from 16,7 to 21,2 %. The ash records were from 9,3 to 12,0 % and neutral detergent fiber from 24,2 to 32,5 %.

Conclusions: All the varieties showed high potential for forage production under the conditions of both altitudinal plateaus. Likewise, the environmental conditions were determinant in the performance of the different genotypes.

Keywords: chemical composition, genotype environment interaction, forage legumes

Introduction

Medicago sativa L. is an important forage legume in agricultural and animal husbandry systems of semiarid zones. It is cultivated in all the regions of the world and adapts to the subtropical, temperate and dry climates (Gu *et al*., 2018). Its importance is due to its production potential and nutritional value to feed livestock (Álvarez-Vázquez *et al*., 2018). It requires a high content of nutrients in the soil, mainly phosphorus, which is lodged in the roots, and is later used by the leaf area, with which the biomass yield is increased (Gu *et al*., 2018; Oñate-Vitteri and Flores-Mariazza, 2019).

The climate conditions of the mountain or Andes zone are very variable, because of the marked seasonality of rain and drought, besides the temperature variations during day and night. The differences among seasons influence the productive conditions of *M. sativa*, which is also shown in the genotypes, which have a different capacity of adaptation to such conditions (Rivas-Jacobo *et al*., 2020). It is known that the characteristics of the environment where the cultivars are developed condition the productive performance of a same species (Rebora *et al*., 2015; Rivas-Jacobo *et al*., 2020).

According to criteria expressed by Wang *et al*. (2021), the continuous production of *M. sativa* cultivars affects the capacity of water storage and fertility of the soil, specifically the phosphorus availability, as well as the relation between organic carbon and total nitrogen, although their quantities are increased. Fu *et al.* (2021) state that temperature and humidity in the soil influence the structures of the edaphic microbial communities and yield of *M. sativa*, depletion of nutrients and soil organic matter.

The nutrient contents for the *M. sativa* crop, especially sodium (Na), nitrogen (N) and potassium (K) are important for the regrowth and development of root nodules of the cultivars (Chen *et al.,* 2020; Elgharably and Benes, 2021).

This forage legume shows a high protein (17,4 to 20,1 %) and ash content (10,38 to 12,31 %), according

Received: 15/10/2021
Accepted: 31/01/2022

How to cite a paper: Cubas-Leiva, Magali Beatriz; Vallejos-Fernández, Luis Asunción; Florián-Lescano, Roy Roger; Carrasco-Chilón, William Leoncio & Alvarez-García, Wuesley Yusmein. Productive evaluation and chemical composition of six genotypes of *Medicago sativa* L. in the Andes of northern Peru. *Pastos y Forrajes*. 45:eI7, 2022.

to Srisaikham and Rupitak (2021). These values of protein, carbohydrates and fiber can be affected by different environmental conditions (Almuhayawi *et al*., 2021). For such reason, the objective of this work was to evaluate the yield, growth rate, plant height and contents of crude protein (CP), ash and neutral detergent fiber (NDF) in six *M. sativa* L. genotypes and on two altitudinal plateaus (AP) of the Andes of northern Peru.

Materials and Methods

Location of the study. The trial was conducted in the Santa Cruz-Cajamarca province, Peru (latitude 06º48’00” “S”, longitude 78º48’00” “W”), from February, 2018, to March, 2019, on two altitudinal plateaus (AP). The first one (AP I), with ranges from 2 300 to 2 800 m.a.s.l., and the second (AP II), located between 2 801 and 3 300 m.a.s.l.

Climate characteristics. The temperature of AP I was 16 ºC and annual rainfall, 878 mm. Regarding AP II, the average temperature was 11,8 ºC and rainfall, 795 mm during the evaluation months.

Experimental design and treatments. A complete randomized design was used, with three repetitions to evaluate the genotypes. The total surface used in the research was 108 m^2. Each experimental unit had an area of 6 m^2 (3 x 2 m). The six *M. sativa* genotypes were randomly distributed in 18 subplots on each AF. The treatments of this experiment were the six *M. sativa* varieties, from New Zealand.

Experiment planting. For planting the experiment the soil characteristics were determined. The pH^+ values were extremely acid (4,2-4,3), with moderate to high organic matter content. For the case of phosphorus, low values were found (P = 5,2-10,4 ppm) and high potassium content (K = 507-738 ppm). Under these conditions, 2,5 t of dolomite lime/ha were applied, in order to neutralize the soil acidity for the installation of the *M. sativa* cultivars. This practice was done one month before the planting, by drilling. Before planting, fertilization was carried out with triple superphosphate and potassium chloride, to achieve the proportion of 0-350 – 80 kg/ha N – P_2O_5 – K_2O, respectively. Irrigation by sprinklers was applied depending on the plant needs, and according to availability, every 15 days approximately, taking into consideration that it was done on the same day on both APs.

Planting was performed in March, 2018, manually, by drilling. After 55 days, the establishment cut was done to control weeds and favor tillering of the crop. Afterwards, the utilization or harvest cuts were carried out, depending on the emergence of regrowth, when it was in phenological status of flowering onset, to utilize better the forage availability and nutritional quality. The weeding works were carried out in each cut, in order to prevent competition for nutrients between *M. sativa* and the weeds.

Samples. Eighteen samples of pastures were collected per each AP. The measurements of plant height were done after each cut, and were determinant for the harvest moment, as well as the control of the phenological status in which the cultivars were. In AP I, six cuts were made throughout the evaluation period. However, in AP II only three could be done. In each sampling per plot, 400 g of *M. sativa* were taken and a precision scale (H.W. KESSELL. S.A., Germany) was used in the field. The samples were sent to the laboratory for their respective analysis.

Chemical composition. The chemical composition of the six varieties was evaluated: analysis of CP, according to AOAC 984.13 (AOAC, 2012), total fat or ethereal extract by AOAC 920.39 (AOAC, 1990) and ash, according to AOAC 942.05 (AOAC, 2000) (Thiex *et al*., 2012). In addition, NDF was analyzed through the method proposed by Van Söest (1991) in the laboratory of soils, waters and forages of the Agricultural Research Station Baños del Inca-Cajamarca.

Plant height. Evaluations of height were made, from the soil level (2 cm) to the maximum height of the cultivar in the quadrant in each experimental unit. A metallic 70-cm ruler was used. Three height measurements were taken in each quadrant and the average was used for the statistical comparison.

Productive yield. It was determined by cutting *M. sativa* in the 30 x 30 cm quadrants (0,09 m^2). The forage samples were put in plastic bags and were identified with an indelible-ink pen for each cultivar. The samples (400 g) were put in refrigerating boxes and transported to the laboratory to determine DM and nutrients, according to the analysis by Weende and Van Söest.

Growth rate. The growth rate was calculated with regards to the biomass obtained during the evaluation period, according to the formula:

$$\text{Growth rate (kg DM/ha/day)} = \frac{\text{Total biomass (kg DM/ha/year)}}{\text{Number of evaluated days}}$$

Thus the quantity of DM/ha in one day, approximately, was recorded.

Statistical analysis. The validation of the fulfillment of the variance homogeneity assumptions through the Kolmogorov-Smirnov test and data normality (Levene) were carried out. The chemical composition was compared among varieties. However, the productive indicators of green forage (GF) and DM (biomass) yield, growth rate and plant height in the six genotypes and their interaction, was done through a variance analysis. The analysis of additive main effects and the multiplicative interaction models, proposed by Hongyu *et al*. (2014). The platform RStudio (Version 1.4.1717) of R Project (R Core Team, 2020) was used. In addition, Person's correlation tests were made to find the relation between plant height and biomass yield. For the comparison of means Duncan's multiple range comparison test was used ($p < 0,05$).

Results and Discussion

Productive performance. AP I ($p < 0,05$) showed better yield (GF and DM), plant height and growth rate than AP II (table 1). This can be due to the different number of cuts that were obtained in each AP. No significant differences were found for the interaction between the AP and the *M. sativa* varieties on the indicators yield, plant height and growth rate. The GF reports per cut were lower than those recorded by Castro-Bedriñana *et al.* (2019), who found values of 18 700 to 33 400 kg GF/ha/cut with the variety Aragón, at an altitude of 3 250 m.a.s.l. These differences can be due to planting density and season, fertilization and the studied altitude.

The variety W 450 showed better DM yield in one year, with 10 483,9 with regards to the variety Stamino (6 215 kg/ha/year^{-1}). The values of W 450 were similar to the ones obtained by Álvarez-Vázquez *et al*. (2018), who indicate that the biomass production is influenced by environmental conditions, especially by the season. However, lower results than those obtained by Rojas-García *et al.* (2016) were achieved, who reported 20 275 kg DM/ha/year, and the ones reached by Sánchez-Gutiérrez *et al.* (2017), who attained values of 4 507,8 kg DM/ha/cut.

Regarding the GF yield (kg/ha/cut), the best cultivars ($p < 0,05$) were W 450, W 350, SW 10, SW 8210 and Hortus 401. The DM yield per cutting in each AP did not show significant differences. In each one the same quantity of biomass per cut was obtained. Nevertheless, the yield per year was higher in AP I, which can be due to the higher number of cuts.

The growth rate in AP I was higher (28,7 kg DM/ha/day) than in AP II (16,76 kg DM/ha/day), probably because of the better climate conditions (Rebora *et al*., 2015; Rivas-Jacobo *et al*., 2020). The variety W 450 reached the highest yield (28,7 kg DM/ha/day in average) and was statistically similar to SW 10, SW 8210 and W 350. For planting these genotypes, similar conditions to the ones in this study should be considered.

Plant height was higher in AP I, which confirms that between 2 300 and 2 800 m.a.s.l., the climate conditions are more favorable for the growth of *M.*

Table 1. Average values of green forage yield, dry matter, plant height and growth rate.

Altitudinal plateau	Green forage, kg		Dry matter, kg		Plant height,	Growth rate,
	Year	Cut	Year	Cut	cm	kg DM/ha/day
AP I	61 450,6^{a}	10 241,8a	10 498^{a}	1 739,9	31,5	28,8^{a}
AP II	24 833,3^{b}	8 277,8b	6 116,3^{b}	2 038,8	28,3	16,8^{b}
Variety						
W 450	56 963^{a}	12 126,5^{a}	10 483,9^{a}	2 402,3^{a}	31,4^{a}	28,8^{a}
SW 10	44 056^{ab}	9 200,6^{ab}	8 588,2^{abc}	1891,9^{abc}	35,5^{a}	23,5^{abc}
SW 8210	42 148^{ab}	8 753,1^{ab}	7 765,6^{abc}	1 713,1^{bc}	33,4^{a}	21,3^{abc}
W 350	48 815^{ab}	10327,2^{ab}	9 627,7^{ab}	2 177,9^{ab}	30,9^{ab}	26,4^{ab}
Stamino 5	30 814^{b}	6 543,2^{b}	6 215,4^{c}	1 409,7^{b}	26,0^{bc}	17,0^{c}
Hortus 401	37 000^{b}	8 608,0^{ab}	7 161,4^{bc}	1770,2^{abc}	22,4^{c}	19,6^{bc}
SE ±	4 250,4	590	624,38	113,68	1,15	1,71
P - value	0,037	0,037	0,031	0,034	<0,001	0.043

Different letters in each column show significant differences (Duncan test, $p < 0,05$).
AP I: ranges from 2 300 to 2 800 m.a.s.l., AP II: ranges between 2 801 and 3 300 m.a.s.l.

sativa. In this regard, it was determined that plant height had a moderate and positive relation with the growth rate and green forage production per cut (r = 0,68). This is important in order to establish the accumulation of aerial biomass and adjust the yield.

For plant height, the values were lower than those reported by Rojas-García *et al.* (2016), who in a study recorded values from 42,0 to 53,0 cm. These authors compared five varieties different from the ones evaluated in this work. They were also lower than the values indicated by Oñate-Vitteri and Flores-Mariazza (2019), who obtained from 50,2 to 78,0 cm, in a study conducted at 2 754 m.a.s.l.

Effect of variability between genotype and environment. The variability was determined for the production of GF, DM, growth rate and plant height (table 2). In the variability for the annual GF production, 76 % corresponded to the environment or AP, 15 % to the varieties (genotype) and 9 % to the interaction between the varieties and the APs. This was determinant to explain that the *M. sativa* varieties achieved different results for the number of cuts. In AP I higher number of harvests was obtained, which proved the superiority of AP I (2 300-2 800 m.a.s.l.).

Plant height responded better to the genotype planted in the plots. This can show that the varieties express their particular characteristics of size and utilization height. Likewise, growth rate was affected by the environment. This corroborates that the daily accumulated biomass yields in the six *M. sativa* varieties showed better response between 2 300 and 2 800 m.a.s.l.

Chemical composition. The CP contents among the cultivars were similar, and were between 16,7 and 21,2 %. These results are very acceptable for feeding dairy cattle in the high Andean zone. Nevertheless, they were lower than the reports by Capacho-Mogollón *et al.* (2018), Contreras *et al.* (2019) and Ison *et al.* (2020), and are similar to the results obtained by Alonzo Griffith and Paniagua Alcaraz (2010), who found values of 20,0 % with different doses of calcareous fertilizers in the soils. The lowest ash concentration corresponded to the cultivar SW 10. The NDF varied from 23,1 to 32,5 %. The highest content (p < 0,001) corresponded to the variety SW 10. On the contrary, the varieties that contributed the lowest NDF concentration were W 450, Hortus 401 and W 350.

It was determined that there were no statistical differences for the chemical composition between the two APs. The variety W 450 was the one with the best productive response, because it achieved the lowest NDF value. This shows that it is a genotype that could have better indicators of digestibility and nutritional contribution for the animals, and the one that would adapt best to the climate conditions that correspond to this study.

Table 2. Variability for the green forage yield, dry matter, plant height and growth rate, %.

Variation source	Green forage		Dry matter		Plant height	Growth rate
	Year	Cut	Year	Cut		
Environment (AP)	75,7	20,6	61,4	12,8	10,2	61,4
Genotype (varieties)	15,4	56,0	26,7	63,4	82,3	26,7
Interaction (genotype x environment)	8,91	23,4	11,9	23,9	7,4	11,9

Table 3. Chemical composition of six *M. sativa* genotypes in the Andes of northern Peru, %.

Genotype	Crude protein	Ash	Neutral detergent fiber
SW 10	16,7	9,3^{b}	32,5^{a}
SW 8210	21,2	11,4^{a}	26,8^{b}
W 450	19,7	10,6^{ab}	23,1^{d}
W 350	18,8	11,4^{a}	25,2^{bcd}
Stamino 5	19,6	11,6^{a}	25,4^{bc}
Hortus 401	20,8	12,0^{a}	24,2^{cd}
SE ±	0,711	0,278	0,772
P - value	0,760	0,025	<0,001

Different letters in each column show significant differences (Duncan p < 0,05).

Conclusions

All the varieties showed high potential for forage production under the conditions of both altitudinal plateaus. Likewise, the environmental conditions were determinant in the performance of the different genotypes.

The highest forage yield and growth rate corresponded to AP I. The cultivars W 450, SW 10, SW 8210 and W 350 obtained the best yield in the two APs. The protein contents varied between 16,7 and 21,2 %, the ash and neutral detergent fiber values from 9,3 to 12,0 and from 23,1 to 32,5 %, respectively.

Acknowledgements

The authors thank the National Innovation Program AGRARA-PNIA, the Association of Animal Husbandry Farmers Pucará, El Trébol of Santa Cruz, Cajamarca and the Support Project of New Zealand to the Peruvian dairy sector, for the logistical support in the development of this research.

Conflict of interests

The authors declare that there is no conflict of interests among them.

Authors' contribution

- Cubas-Leiva, Magali Beatriz. Conception of the research work, development of the experimental stage, preparation of samples for the laboratory, drying and laboratory analyses.
- Vallejos-Fernández, Luis Asunción. Conception of the research work, development of the experimental stage and management of funds for the sample analysis.
- Florián-Lescano, Roy Roger. Conception of the research work and development of the experimental stage.
- Carrasco-Chilón, William Leoncio. Manuscript writing and conception of the research work..
- Alvarez-García, Wuesley Yusmein. Contributed to the project writing and revision of the database; besides conducting the statistical analysis, tabulation of the data and search of literature and final revision of the manuscript.

Bibliographic references

Almuhayawi, M. S.; Hassan, A. H. A.; Al Jaouni, S. K.; Alkhalifah, D. H. M.; Hozzein, W. N.; Selim, S. *et al.* Influence of elevated CO_2 on nutritive value and health-promoting prospective of three genotypes of Alfalfa sprouts (*Medicago sativa*). *Food Chem*. 340:128147, 2021. DOI: https://doi.org/10.1016/j.foodchem.2020.128147.

Alonzo-Griffith, L. A. & Paniagua-Alcaraz, P. L. Efectos de dosis de calcáreo sobre el comportamiento productivo y calidad de la alfalfa. *Investig. Agrar.* 12 (1):35-39. https://www.agr.una.py/revista/index.php/ria/article/view/17, 2010.

Álvarez-Vázquez, P.; Hernández-Garay, A.; Mendoza-Pedroza, S. I.; Rojas-García, A. R.; Wilson-García, Claudia Y. & Alejos-de la Fuente, J. I. Producción de diez variedades de alfalfa (*Medicago sativa* L.) a cuatro años de establecidas. *Agrociencia*. 52 (6):841-851. http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S1405-31952018000600841&lng=en&tlng=es, 2018.

AOAC. *Ash of animal feed. Official methods of analysis*. 17th ed. Gaithersburg, USA: Association of Official Analytical Chemists, 2000.

AOAC. *Fat (crude) or ether extract in animal feed. Official methods of análisis.* 15th ed. Washington: Association of Official Analytical Chemists, 1990.

AOAC. *Nitrogen in meat. Kjeldahl method. Official methods of analysis.* 19th ed. Virginia, USA: AOAC International, 2012.

Capacho-Mogollón, A. E.; Flórez-Delgado, D. F. & Hoyos-Patiño, J. F. Biomasa y calidad nutricional de cuatro variedades de alfalfa para introducir en Pamplona, Colombia. *Cien. Agri.* 15 (1):61-67, 2018. DOI: http://doi.org/10.19053/01228420.v15.n1.2018.7757.

Castro-Bedriñana, J.; Chirinos-Peinado, Doris & Lara-Schwartz, Percy. Evaluación del compost de guano de pollo en el rendimiento y calidad nutricional de la alfalfa en la sierra central del Perú. *Rev. investig. vet. Perú*. 30 (4):1562-1568, 2019. DOI: https://dx.doi.org/10.15381/rivep.v30i4.15756.

Chen, S.; Li, Xiang; Liu, Xin; Wang, N.; An, Qi; Ye, Xi M. *et al.* Investigation of chemical composition, antioxidant activity, and the effects of alfalfa flavonoids on growth performance. *Oxid. Med. Cell. Longev.* 2020:8569237, 2020. DOI: https://doi.org/10.1155/2020/8569237.

Contreras, J. L.; Cordero, A. G.; Curasma, J.; Thimothée, J. A. & Del-Solar, J. Environmental influence on the nutritional value of alfalfa (*Medicago sativa* L.) in peruvian Andes. *Compend. cienc. vet*. 9 (1):7-14, 2019. DOI: https://doi.org/10.18004/compend.cienc.vet.2019.09.01.07-14.

Elgharably, A. & Benes, Sharon. Alfalfa biomass yield and nitrogen fixation in response to applied mineral nitrogen under saline soil conditions. *J. Soil Sci. Plant*. 21:744–755 2021. DOI: https://doi.org/10.1007/s42729-020-00397-6.

Fu, B.; Li, Z.; Gao, Xueqin; Wu, L.; Lan, Jian & Peng, W. Effects of subsurface drip irrigation on alfalfa (*Medicago sativa* L.) growth and soil microbial community structures in arid and semi-arid areas of northern China. *Appl.*

Soil Ecol. 159:103859, 2021. DOI: https://doi.org/10.1016/j.apsoil.2020.103859.

Gu, Y.-J.; Han, C.-L.; Fan, J.-W.; Shi, X.-P.; Kong, M.; Shi, X.-Y. *et al*. Alfalfa forage yield, soil water and P availability in response to plastic film mulch and P fertilization in a semiarid environment. *Field Crops Res*. 215:94-103, 2018. DOI: https://doi.org/10.1016/j.fcr.2017.10.010.

Hongyu, K.; Garcıa-Peña, Marisol; Araujo, L. B. de & Dias, C. T. dos S. Statistical analysis of yield trials by AMMI análisis of genotype×environment interaction. *Biometrical Letters*. 51 (2):89-102, 2014. DOI: https://doi.org/10.2478/bile-2014-0007.

Ison, K. A. D.; Benvenutti, M. A.; Mayer, D. G.; Quigley, S. & Barber, D. G. Maximising lucerne (*Medicago sativa*) pasture intake of dairy cows: 2—the effect of post-grazing pasture height and mixed ration level. *Animals, Basel*. 10 (5):904, 2020. DOI: https://doi.org/10.3390/ani10050904.

Oñate-Viteri, W. & Flores-Mariazza, E. Comportamiento agronómico de tres variedades de alfalfa (*Medicago sativa* L.) con diferentes dosis de fertilización fosfatada. *Pastos y Forrajes*. 42 (2):125-132. http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S0864-03942019000200125&lng=es&tlng=es, 2019.

R Core Team. *R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. http://www.r-project.org/index.html, 2020.

Rebora, Cecilia; Barros, A.; Ibarguren, Leandra; Bertona, Alejandra; Antonini, C. & Arenas, F. Efecto del grado de reposo invernal de alfalfa (*Medicago sativa* L.) sobre el rendimiento de heno en el oasis norte de Mendoza. *Rev. FCA UNCUYO*. 47 (2):43-51. https://revistas.uncu.edu.ar/ojs/index.php/RFCA/article/view/3302, 2015.

Rivas-Jacobo, M. A.; Herrera-Haro, J. G.; Hernández-Garay, A.; Vaquera-Huerta, H.; Alejos-de-la-Fuente, J. I. & Cadena-Villegas, S. Yield of five varieties of lucerne during four years of evaluation. *Rev. Mex. Cienc. Agríc*. 11 (n. spe24):141-152, 2020. DOI: https://doi.org/10.29312/remexca.v0i24.2365.

Rojas-García, A. R.; Hernández-Garay, A.; Cansino, S. J.; Maldonado-Peralta, María de los Á.; Mendoza-Pedroza, S. I.; Álvarez-Vázquez, P. *et al.* Comportamiento productivo de cinco variedades de alfalfa. *Rev. Mex. Cienc. Agríc*. 7 (8):1855-1866. http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S2007-09342016000801855&lng=es&tlng=es, 2016.

Sánchez-Gutiérrez, R. A.; Servin-Palestina, M.; Gutiérrez-Bañuelos, H. & Serna-Pérez, A. Eficiencia en el uso del agua de variedades de alfalfa (*Medicago sativa* L.) con sistema de riego subsuperficial. *Rev. mex. de cienc. pecuarias*. 8 (4):429-435, 2017. DOI: https://doi.org/10.22319/rmcp.v8i4.4255.

Srisaikham, Supreena & Rupitak, Quanjai. A preliminary study on growth, yield and nutritive value of four varieties of alfalfa and the utilization of alfalfa dehydrated pellets in a total mixed ratio in meat goat diet. *CMUJ. Nat. Sci*. 20 (1):e2021003, 2021. DOI: https://doi.org/10.12982/CMUJNS.2021.003.

Thiex, Nancy J.; Novotny, L. & Crawford, A. Determination of ash in animal feed: AOAC official method 942.05 revisited. *J. AOAC Int*. 95 (5):1392-1397, 2012. DOI: https://doi.org/10.5740/jaoacint.12-129.

Van Soest, P. J.; Robertson, J. B. & Lewis, B. A. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci*. 74 (10):3583-3597, 1991. DOI: https://doi.org/10.3168/jds.S0022-0302(91)78551-2.

Wang, Linlin; Xie, J.; Luo, Zhuzhu; Niu, Y.; Coulter, J. A.; Zhang, R. *et al*. Forage yield, water use efficiency, and soil fertility response to alfalfa growing age in the semiarid Loess Plateau of China. *Agric. Water Manag*. 243:106415, 2021. DOI: https://doi.org/10.1016/j.agwat.2020.106415.