

Nutritional quality of amaranth (*Amaranthus*) silage in response to forage airing and addition of lactic bacteria

Calidad nutricional de amaranto (*Amaranthus*) ensilado en respuesta a la aireación del forraje y a la adición de bacterias lácticas

María Fany Zubillaga ^{1,2}, Julián Agustín Repupilli ¹, Patricia Boeri ^{1,2},
Juan Agustín Servera ^{1,3}, Juan José Gallego ³, Lucrecia Piñuel ^{1,2*}

Originales: *Recepción*: 11/05/2023 - *Aceptación*: 29/02/2024

ABSTRACT

Climate change is reducing forage availability for ruminants. Previous studies in Northern Patagonia, Argentina, have demonstrated the adaptation of the amaranth crop to these agroclimatic conditions under irrigation. Moreover, this crop is used as forage in marginal areas of the world, given its outstanding productive and nutritional qualities. The objective of this study was to evaluate the nutritional quality of amaranth silage in response to previous wilting and the addition of lactic acid bacteria. The crop was harvested at the milky grain stage and ensiled in experimental microsilos for 60 days. Before ensiling, different treatments (wilting and addition of lactic acid bacteria) were applied. Parameters related to nutritional quality were evaluated, including ash, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), dry matter digestibility (DMD), and metabolizable energy (ME). Simultaneous treatment with air and the addition of lactic acid bacteria before ensiling resulted in the best nutritional quality characteristics of the silage. The most significant results were protein value of 12.7%, 41.1% NDF and 19.1% FDA. The DM and ME were 74% and 2.67 Mcal/kg, respectively. Thus, amaranth silage can be considered an alternative conserved forage for animal feed in this region.

Keywords

nutritional value • amaranth • silage • inoculation • quality

-
- 1 Universidad Nacional de Río Negro. Sede Atlántica. Don Bosco y Leloir s/n. Viedma (8500). Río Negro. Argentina. *lpinuel@unrn.edu.ar
 - 2 Universidad Nacional de Río Negro (UNRN-CONICET). CIT-RIO NEGRO Sede Atlántica. Don Bosco y Leloir s/n. Viedma (8500). Río Negro. Argentina
 - 3 Instituto Nacional de Tecnología Agropecuaria (INTA). Estación Experimental Valle Inferior de Río Negro. Ruta Nac. N° 3 km 971 - Camino 4 IDEVI (8500) Viedma. Río Negro. Argentina.

RESUMEN

El cambio climático está reduciendo la disponibilidad de forraje para los rumiantes. Estudios previos en la Patagonia Norte Argentina demuestran la adaptación del cultivo de amaranto a estas condiciones agroclimáticas bajo riego. Sin embargo, este cultivo se utiliza como forraje en zonas marginales del mundo, dadas las destacadas cualidades productivas y nutricionales del cultivo de amaranto. El objetivo de este estudio fue evaluar la calidad nutricional del ensilado de amaranto en respuesta al oreado previo del forraje y a la adición de bacterias lácticas. El cultivo se cosechó en el estado grano lechoso y se ensiló en microsilos experimentales durante 60 días. Antes del ensilado, se llevaron a cabo diferentes tratamientos (oreado y adición de bacterias lácticas). Se evaluaron parámetros relacionados con la calidad nutricional: cenizas, proteína cruda (PC), Fibra Detergente Neutro (FDN), Fibra Detergente Ácido (FDA), digestibilidad de la materia seca (DMS) y energía metabolizable (EM). El tratamiento simultáneo de oreado y adición de bacterias lácticas antes del ensilado dio lugar a las mejores características de calidad nutricional del ensilado obtenido. Los resultados más importantes bajo estas condiciones fueron valores de proteína del 12,7%, FDN del 41,1% y FDA del 19,1%. La DMS y la EM fueron del 74% y 2,67 Mcal/kg, respectivamente. Así, el ensilaje de amaranto puede ser considerado como una alternativa forrajera conservada para la alimentación animal de la región.

Palabras claves

valor nutricional • amaranto • ensilado • inoculación • calidad

INTRODUCTION

The projected growth in global food demand has led to an increased focus on underutilized crops, with the potential to improve global food security and mitigate the adverse effects of climate change. Animal production in the Lower Rio Negro Valley is limited by a seasonal lack of forage. Feed and conserved fodder, such as silage, ensures forage quality and quantity. The main silage resource in the area is corn, with a biomass yield of over 30 t/ha and a protein content of 8%. However, its cultivation requires 900 mm of water and 300 kg/ha of nitrogen (27). *Amaranthus cruentus* cv Mexicano has shown adaptability to the local environmental conditions with yields of 21 t/ha, needing 800 mm and 150 kg/ha of water and nitrogen requirements, respectively (39, 40, 41). Therefore, amaranth could be an alternative forage resource for the region given its high biomass production and low management requirements. Seguí *et al.* (2013) stated that amaranth shows high rumen degradability when used as animal feed, either by direct grazing or as conserved forage. The nutritive value of this forage varies according to the developmental stage, standing out for its high crude protein content (8-29%), low lignin values (1.7-7.3%), and high *in vitro* digestibility (59-79%) (23, 28). However, high moisture and protein content could negatively affect the ensiling process. According to Borreani *et al.* (2018), moisture content produces effluents that reduce soluble carbohydrates, whereas high protein values in the forage have a buffering capacity and prevent pH decrease (<5.0) in the silage. A common practice to decrease forage moisture is to wilt the forage in the field for 24-48 hours under good weather conditions (no risk of rain) and with minimal mechanical treatment. This increases dry matter, resulting in a higher concentration of soluble carbohydrates that favors the fermentation process and nutritive value (37). The use of additives, such as bacterial inoculants, could favor a rapid decrease in pH, improving the conservation and nutritive value of silage (4, 32). Among the most commonly used inoculants are lactic acid bacteria (LAB), which ferment carbohydrates into lactic acid, acidifying the silage and inhibiting growth of undesirable bacteria (24), thus improving the fermentation and aerobic stability of the final product. Although silage techniques have been studied for this crop, different results depend on variety, cutting time, processing methodology, and place of origin (16, 20, 28, 29). In South America, there is no information on amaranth as fodder or its conservation in silage. In this study, we hypothesized that wilting and LAB inoculation would improve

fermentation quality of amaranth forage. The objective of this study was to evaluate the nutritional quality of amaranth silage in response to previous wilting of forage and addition of lactic acid bacteria.

MATERIALS AND METHODS

Site location, climatic conditions, and forage production

Field experiments were conducted at the INTA-Estación Experimental Agropecuaria Valle Inferior del Río Negro (40°48' S, 63°05' W; 4 m). This area in Patagonia, Argentina, has an irrigation system that covers 24,000 ha. During the growth period of amaranth in 2019 (November to April), rainfall was 186 mm and the average temperature was 19°C. The physicochemical characteristics of the upper 50 cm of the experimental loam soil were: pH (8.20); electrical conductivity (1.2 mmhos cm⁻¹); organic matter (3.8%); total nitrogen (0.18%); N-NO₃ (24.60 mg kg⁻¹); P (Olsen, 16.60 mg kg⁻¹); S (14.7 mg kg⁻¹ as a SO₄⁼); Ca (8.230 mg kg⁻¹); Mg (1.170 mg kg⁻¹); sodium-adsorption ratio (1.83). The INTA laboratory performed the soil characterization.

The cultivar evaluated was *A. cruentus* cv Mexicano, sown in rows with a horticultural seeder at the end of spring (November 20th). The sowing area was 70 m² (10 furrows 0.7 m wide × 10 m long). Weeding was performed manually when the plants reached 20-30 cm in height; thinning was performed by hand, leaving ten plants m⁻². Furrow irrigation was applied according to soil moisture retention curve before reaching the permanent wilting point, with a total lamina of 800±50 mm. Fertilization with granulated urea (46% N), with a nitrogen (N) dose of 90 ha⁻¹, was carried out in two stages; on plants 60 cm high and at bloom.

Treatment of plant material before the silage process

The forage was cut at advanced flowering stage (between milky-pasty grains), corresponding to a chronological time of 123 days or 1627 growing degree days, with dry matter (DM) values above 20%. Amaranth plants were manually cut 50 cm above the soil surface (26) and then wilted in the field for 24 hours before chopping the material. The plants were chopped using a Thomas Willey mill until they reached a size of 1-3 cm. The chopped material was then treated with lactic acid bacteria (LAB) under conditions recommended by the manufacturer using an atomizer. (Bemix Plus® 2% w/v). Four different types of silage were obtained: unwilted, ensiled amaranth (UWAE); unwilted, ensiled amaranth with added lactic acid bacteria (UWAEEL); wilted, ensiled amaranth (WAE); and wilted, ensiled amaranth with added lactic acid bacteria (WAEEL).

Ensiling process

The silage was prepared at laboratory scale using 110mm PVC tubes 30 cm long, known as experimental microsilage. The filling of the experimental microsilage was performed by placing the plant material in compacted layers with a hydraulic press (140 kgf) ensuring homogeneous compaction. The tubes were capped and sealed to achieve anaerobic conditions (four replicates for each forage type). The experimental microsilage was maintained under environmental conditions for 60 days.

Characterization and chemical analyses of the forage and silage types

Chop size: A fresh sample (100 g) of non-ensiled plant material was measured using a Vernier caliper and grouped according to length. After the ensiling period, the average temperature was determined with an infrared thermometer (AMPROBE IR-710) and a sample (50 g) was collected at a depth of 15 cm from each silo. All samples were frozen at -18°C until use for quality determination. An aqueous extract (1:4) was made and left to rest for 1 h at room temperature, after which pH was measured with a pH meter (Foodcare HI99161) (10). Dry matter content (% DM) was determined according to the AOAC (2000). The samples were ground using a grinder (ARCANO®). Ash content, crude protein (CP%), Neutral Detergent Fiber (NDF %), and Acid Detergent Fiber (ADF %) were determined following the methodology proposed by the AOAC (2000). The dry matter digestibility (% DMD) was estimated using the predictive equation proposed by Rohweder *et al.* (1978). Metabolizable

Energy (ME) was determined using the following formula: $EM: 3.61 \cdot DMD$ according to Di Marco (2011). All measurements were performed in triplicate.

Statistical analysis

A completely randomized block design was used, with four treatments and four replicates for each treatment. Pre-ensiling quality variables were analyzed using simple ANOVA (WA-C; UWA-C). Silage quality parameters were analyzed by double ANOVA with the following sources of variation: wilting (UWAE; WAE), inoculation (UWAEL; WAEL), and their interaction as main effects (2×2 factorial design). The T° and pH showed no interaction, while DM, CP, ash, NDF, and SDF were analyzed by simple ANOVA to evaluate the effect of wilting and addition of lactic acid bacteria separately. Comparisons of means were made using Fisher's least significant difference (LSD) test at 5%. Statistical analyses were performed using InfoStat (8).

RESULTS AND DISCUSSION

Pre-ensiling characteristics of forage

Visual differences in coloration between UWA-C and WA-C were observed in the chopped plant material (data not shown). The latter had an opaque yellow-olive color, whereas UWA-C was bright olive-green, which could be associated with the loss of water during the wilting process. In addition, drying plant material in the sun results in the formation of indigestible protein-carbohydrate complexes called Maillard products. The DM values are presented in table 1. Knowing the proper DM content of forage at the pre-ensiling stage is essential to achieve efficient compaction and an anaerobic environment while preventing growth of undesirable microorganisms. DM content of 30-35% is usually recommended to obtain high-quality amaranth silage (14). Krawutschke *et al.* (2013) achieved an optimum DM content of 30-35 % when wilting red clover (*Trifolium pratense L.*), which like amaranth is difficult to ensile due to a lower water-soluble carbohydrate concentration (WSC), higher buffering capacity (BC) and lower dry matter (DM) content at harvest. In amaranth, wilting (WA-C) resulted in dry matter values that reached the recommended values for silage; therefore, wilting could favor a higher concentration of soluble carbohydrates and a lower concentration of lactic acid (21).

Table 1. Nutritional parameters of unwilted amaranth control (UWA-C) and wilted amaranth control (WA-C).

Tabla 1. Parámetros nutricionales del forraje de amaranto no oreado control (UWA-C) y forraje de amaranto oreado control (WA-C).

	DM %	CP %	NDF %	ADF %	DMD %	ME Mcal/kg DM
UWA-C	20.3 b	15.8 a	46.1 b	26.4 b	68.3	2.5
WA-C	34.8 a	14.1 b	51.7a	27.6a	67.4	2.4
Standard error	0.36	0.09	0.58	0.27		
<i>p-value</i>	0.0001	0.0007	0.0001	0.0025		

Dry matter (DM); Crude Protein (CP), Neutral Detergent Fiber (NDF); Acid Detergent Fiber (ADF), Dry Matter Digestibility (DMD), Metabolizable Energy (ME) associated with the quality of the forage before silage.

Values of the same variable followed by the same letter are not statistically different according to Fisher's LSD test ($p > 0.05$).

Materia Seca (MS); Proteína Cruda (PC), Fibra Detergente Neutra (FDN); Fibra Detergente Ácida (FA), Digestibilidad de la Materia Seca (DMS), Energía Metabolizable (EM) asociados a la calidad del forraje antes del ensilaje.

Los valores de las mismas variables seguidos de la misma letra no son estadísticamente diferentes según la prueba LSD de Fisher ($p > 0,05$).

On the other hand, table 1 (page 120), shows the nutritional parameters of the material before the ensiling process and statistically significant differences ($p > 0.001$) were observed between the UWA-C and WA-C treatments. Forage total N determines the total amount of N available for animal consumption. In this study, the %CP of UWA-C was higher than that of WA-C, possibly because the drying process favors losing nonstructural carbohydrates, volatile organic substances, and protein degradation. This results in amino acids with higher solubility that can be removed from plant tissues, and although this process can significantly decrease the %CP, it varies depending on the drying method. N decreased by only 5% in this case because of wilting conditions. WA-C had the highest fiber content (NDF and SDF), possibly because exposure of the material to ultraviolet radiation (wilting) decreased forage soluble components. In this study, NDF content ranged from 46.1% to 51.7%, and FAD content ranged from 26.4% to 27% when amaranth plants were wilted. Fiber components, such as NDF, FAD, and lignin, are generally inert during plant respiration, but their concentrations increase because of a decrease in oxidized soluble compounds. Therefore, slow-drying methods are expected to result in higher proportions of NDF and FAD. The digestibility of UWA-C was significantly higher ($p > 0.001$) than that of WA-C, mainly given by the lower fiber content of the undried plant material.

On the other hand, the size of the plant material obtained in this study before ensiling was between adequate ranges (1-3 cm), coinciding with the recommendations of Citlak and Kilic (2020).

Determination of parameters related to fodder processing and conservation

A double ANOVA determined that the wilting and inoculation treatments did not affect the temperature and pH variables. However, as shown in table 2, significant differences in T° were observed with the wilting effect, where the minimum and maximum values (UWAE and WAE, respectively) were recorded, but there were no differences in this parameter after LAB addition. Temperature affected silage fermentation, and the best results were obtained with a moderate T between 20 and 30°C. For corn silage, Zhou *et al.* (2019), reported that increasing storage temperature from 5°C to 25°C did not affect fermentation profiles of most biochemical parameters or bacterial and fungal populations. In the present study, the low temperatures observed when opening the experimental microsilage could be related to low ambient temperature at the study site.

Table 2. Parameters related to fodder processing and conservation.

Tabla 2. Parámetros relacionados con el procesado y conservación del forraje.

Treatment	T° (°C)	pH
UWAE	15.95 ± 1.42 b	4.6 ± 0.13b
WAE	18.10 ± 1.17 a	4.9 ± 0.22a
UWAEEL	16.05 ± 1.86 ab	4.5 ± 0.04b
WAEEL	17.50 ± 0.71 ab	4.6 ± 0.71b
Standard error	0.68	0.05
<i>p</i> -valor	0.6158	0.0595

Temperature (T°), and pH of the experimental microsilage at the moment of opening for unwilted amaranth ensiled (UWAE); unwilted amaranth ensiled with added lactic acid bacteria (UWAEEL); wilted amaranth ensiled (WAE); wilted amaranth ensiled with added lactic acid bacteria (WAEEL).

Values of the same variable followed by the same letter are not statistically different according to Fisher's LSD test ($p > 0.05$).

Temperatura (T°), y pH de los microsilos experimentales en el momento de apertura. Amaranth ensilado sin orear (UWAE); amaranth ensilado oreado (WAE); amaranth ensilado sin orear con adición de bacterias lácticas (UWAEEL); amaranth ensilado oreado con adición de bacterias lácticas (WAEEL).

Los valores de las mismas variables seguidos de la misma letra no son estadísticamente diferentes según la prueba LSD de Fisher ($p > 0,05$).

When anaerobic fermentation occurs in the silage process, lactic acid bacteria in the forage are desirable because they rapidly lower the pH, achieve rapid stabilization, and maintain the characteristics of the ensiled material (9, 36). The pH (average of 4.65) observed for all silages was within the established values for good-quality silages, which is comparable with the values reported for different amaranth varieties, harvest stages, and place of cultivation (26). Several factors may be responsible for silage having a higher than normal pH (~4.0), including buffering capacity provided by high protein content (17). In UWAE and WAEL, the use of bacterial inoculants slightly increased acidity of the experimental microsilage. Similar results have been obtained for corn, oats, and amaranth silages (4, 20). In alfalfa, a forage with high buffering capacity, wilting pretreatment, and a pH of 5.19 was reported after 60 days of ensiling (11). In this study, silage with pre-wilting and higher DM (WA-C, table 1, page 120) showed a slight increase in T° and pH; however, the silage obtained with wilted forage and addition of lactic acid bacteria (WAEL) only showed a decrease in pH.

Other authors have reported that lactic acid bacteria in temperate to cold environments favor a lower pH and rapid production of desirable metabolites, such as lactic acid, accelerating fermentation, and better-conserving silage nutrients over a wide range of growth temperatures (4, 13).

Nutritional quality of ensiled plant material

For the DM, CP, Ash, NDF, and ADF variables, the double ANOVA detected interactions between sources of variation (wilting and inoculation with LAB); therefore, the results are presented separately (table 3). When DM was determined after the ensiling process, values of approximately 20% were observed in the unwilted plant material (UWAE and UWAE) and values approached 34% in the wilted material (WAE and WAEL). These values are similar to those obtained before ensiling (table 1, page 120). Good fermentation results in DM losses of less than 10% (3), and the dry matter reduction was <1% in our case.

Table 3. Parameters related to nutritional quality.
Tabla 3. Parámetros relacionados con la calidad nutricional.

Without LAB	DM (%)	CP (%)	ASH (%)	NDF (%)	ADF (%)	DMD (%)	ME (Mcal/kg DM)
UWAE	20.24b	11.14b	14.19a	48.58a	26.36a	68.37	2.47
WAE	34.47a	13.43a	14.31a	44.07b	24.30b	69.97	2.53
Standard error	0.58	0.12	0.01	0.06	0.03		
<i>p-value</i>	0.0001	0.0001	0.0603	0.0001	0.0001		
With LAB	DM (%)	CP (%)	ASH (%)	NDF (%)	ADF (%)	DMD (%)	ME (Mcal/kg DM)
UWAE	20.66b	13.32a	15.23a	48.80a	26.61a	68.17	2.46
WAEL	33.59a	12.71b	12.87b	41.11b	19.12b	74	2.67
Standard error	0.25	0.56	0.03	0.04	0.28		
<i>p-value</i>	0.0001	0.0299	0.0001	0.0001	0.0001		

Dry Matter (DM); Crude Protein (CP), Neutral Detergent Fiber (NDF); Acid Detergent Fiber (ADF), Digestibility of Dry Matter (DMD), Metabolizable Energy (ME) of ensiled plant material. Unwilted amaranth ensiled (UWAE); wilted amaranth ensiled (WAE); unwilted amaranth ensiled with added lactic acid bacteria (UWAE); wilted amaranth ensiled with added lactic acid bacteria (WAEL).

Values of the same variable followed by the same letter are not statistically different according to Fisher's LSD test ($p > 0.05$).

Materia Seca (MS); Proteína Cruda (PC), Fibra Detergente Neutra (FDN); Fibra Detergente Ácida (FDA), Digestibilidad de la Materia Seca (DMS), Energía Metabolizable (EM) del material vegetal ensilado. Ensilado de amaranto sin orear (UWAE); ensilado de amaranto oreado (WAE); ensilado de amaranto sin orear con adición de bacterias lácticas (UWAE); ensilado de amaranto oreado con adición de bacterias lácticas (WAEL).

Los valores de las mismas variables seguidos de la misma letra no son estadísticamente diferentes según la prueba LSD de Fisher ($p > 0,05$).

High protein content is generally associated with higher forage quality, and protein content has been reported to vary from 11.5 to 14% in amaranth silage (16). These variations could be associated with on-site environmental conditions and agronomic practices affecting nutritional composition (39). Table 3 (page 122) shows protein content of the different silages used in this study, with values within the mentioned range. Thus, UWAE had the lowest value (11.14%) for this variable, whereas WAE had the highest value (13.43%). A decrease in protein content of the ensiled material was observed when compared with the non-silage forage (WA-C and UWA-C). The greatest decrease in protein content was observed in UWAE (26% and 12% in UWAE and UWAEEL, respectively); however, these decreases were smaller in previously wilted forage (7% WAE and 10% WAEL). In general, a higher osmotic pressure results from higher dry matter (DM) content, which affects plant enzymatic activity and reduces proteolytic capacity (16, 30). This leads to a decrease in soluble protein (CP) and an increase in the insoluble protein fraction, although the latter is still potentially degradable in wilted silages (34, 35).

In contrast, the addition of LAB to unwilted forage decreased proteolysis by 54% and CP loss decreased. However, in the case of WAEL, the addition of LAB did not decrease proteolysis, indicating an interaction between wilting and the addition of LAB, reflected in a decrease in CP. These results agree with those published by Abbasi *et al.* (2018), who observed decreases in protein content in relation to fresh material of 9%, 10%, and 7.5% in ensiled forage, silage with lactic acid bacteria, and wilted forage, respectively.

Ash contents of the different experimental microsilages is listed in table 3 (page 122). The silages with BL (UWAEEL and WAEL) had the highest and lowest ash values, respectively. However, silages without the addition of lactic acid bacteria (UWAE-WAE) did not show statistically significant differences ($p>0.05$), and the wilting process did not affect ash content. Amaranth species are characterized by high mineral content that can vary according to variety, harvest time, climate, soil, and crop management (39). The ash values obtained were within the range of those published by other authors for amaranth silage, which provides more minerals for livestock (25, 28). Seguí *et al.* (2013) reported that the major mineral in amaranth is Ca, generally found as calcium carbonate, and a fermentation process can favor the forage buffering capacity to the detriment of its acidification.

Fibers in a feed consist of structural carbohydrates in the cell walls and soluble or nonstructural carbohydrates. Concerning fiber content of fresh chopped forages (UWA-C and WA-C), the ensiling process reduced the NDF and ADF values, in agreement with previous reports (16, 20, 25). Fiber values in the silages obtained (table 3, page 122) were within the ranges described in the literature for this crop (NDF: 28-47.7% and ADF: 26-31%) (14, 21). ADF and NDF showed statistically significant differences due to the effect of wilting, and a decrease was observed as a consequence. However, the addition of LAB further favored this decrease in ADF and NDF. Similar results have been reported for amaranth under similar conditions (wilting and addition of LAB) (1). However, in our study on unwilted silages, no such decrease was observed. The higher moisture content and lower temperature of the experimental microsilage in UWAE and UWAEEL may have limited the fermentation process and decreased the action of cellulolytic bacteria responsible for the reduction in the fibrous fraction (14, 38). Fiber content is directly related to digestibility and influences the rate at which feed passes through the digestive tract of an animal (18). Digestibility values for UWAE and UWAEEL mirrored those observed for FDN and FAD, and the addition of LAB did not increase this parameter. However, WAE and WAEL showed higher digestibility and metabolizable energy values (table 3, page 122). A synergistic effect was observed between wilting and the addition of LAB, achieving silage with a digestibility of 74.02%. This silage would be classified as good quality according to Di Marco's classification (7), whereas the rest of the silages would be of medium quality. Regarding the ME of WAEL (2.67 Mcal/k DM), an 8% increase was observed with respect to the non-silage plant material (WA-C). This energy value can be expressed as 11.17 MJ/k DM and is within the range determined for silages of other amaranth species (1, 29). Based on the results obtained in this study, we can conclude that WAEL presented the best organoleptic, conservation, and nutritional characteristics among the four silages evaluated. By comparing the quality of this silage with the values described for crops traditionally used in this type of conservation technique (table 4, page 124), we can infer that it is comparable to the quality of corn silage.

Table 4. Comparison of nutritional quality parameters of the main crops used as fodder.
Tabla 4. Comparación de los parámetros de calidad nutricional de los principales cultivos utilizados como forraje.

	DM (%)	pH	CP (%)	Ash (%)	ADF (%)	NDF (%)	DMD (%)	ME	Reference
Corn	36	3.5	8.5	5.5	15.8	36.6	75.5	2.7	(21)
Sorghum	33.7	4	6.9	8.7	30.7	55	66.3	2.4	(6)
Amaranth	33.5	4.6	12.7	12.9	19.1	41.1	74	2.7	This work

Dry Matter (DM); pH; Crude Protein (CP), Ashes; Acid Detergent Fiber (ADF), Neutral Detergent Fiber (NDF); Digestibility of Dry Matter (DMD), Metabolizable Energy (ME).

Materia seca (MS); pH; Proteína Cruda (PC), Cenizas; Fibra Detergente Ácida (FDA); Fibra Detergente Neutra (FDN); Digestibilidad de la Materia Seca (DMS), Energía Metabolizable (EM).

Likewise, it is evident that amaranth protein content did not affect the ensiling process, and its digestibility was comparable to that of corn silage. Regarding corn biomass production for silage, previously reported values in the Lower Rio Negro Valley vary between 16-34 Tn DM/ha, depending on the hybrid and management (19). In this sense, amaranth yields (7.8-21 Tn DM/ha) were comparable to those of corn (39).

CONCLUSION

Wilting and inoculation of amaranth forage with lactic acid bacteria before ensiling resulted in silage with nutritional characteristics (crude protein percentage, fiber content, dry matter digestibility, and metabolizable energy) that can be classified as a high-quality component of animal diets. The practice of ensiling amaranth as an alternative for conserving forage can be considered viable in semi-arid regions such as Patagonia. However, further research under *in vivo* conditions is required, especially regarding animal responses according to category and species, as well as the exploration of combinations with other ingredients to achieve complementarity and a better balance of nutrients and energy.

REFERENCES

1. Abbasi, M.; Rouzbehan, Y.; Rezaei, J.; Jacobsen, S. E. 2018. The effect of lactic acid bacteria inoculation, molasses, or wilting on the fermentation quality and nutritive value of amaranth (*Amaranthus hypochondriacus*) silage. *Journal of animal science*. 96(9): 3983-3992. <https://doi.org/10.1093/jas/sky257>.
2. AOAC. Official Methods of Analysis. 2000. 17th ed. The Association of Official Analytical Chemists Gaithersburg MD USA.
3. Borreani, G.; Tabacco, E.; Schmidt, R. J.; Holmes, B. J.; Muck, R. E. 2018. Silage review: Factors affecting dry matter and quality losses in silages. *Journal of Dairy Science*. 101(5): 3952-3979.
4. Chen, L.; Bai, S.; You, M.; Xiao, B.; Li, P.; Cai, Y. 2020. Effect of a low temperature tolerant lactic acid bacteria inoculant on the fermentation quality and bacterial community of oat round bale silage. *Animal Feed Science and Technology*. 269: 114669. <https://doi.org/10.1016/j.anifeedsci.2020.114669>.
5. Citlak, H.; Kilic, U. 2020. Innovative Approaches in Covering Materials Used in Silage Making. *International Multilingual Journal of Science and Technology*. 5: 2046-2050. <http://www.imjst.org/wp-content/uploads/2020/12/IMJSTP29120390.pdf>
6. De León, M.; Giménez, R. A. 2011. Ensilajes de sorgo y maíz: rendimiento, composición, valor nutritivo y respuesta animal. https://inta.gob.ar/sites/default/files/scripttmp-ensilajes_de_sorgo_y_maz_rendimiento_composicin_va.pdf (February 2018).
7. Di Marco, O. 2011. Estimación de calidad de los forrajes. *Producir XXI*. 20(240): 24-30. https://www.produccion-animal.com.ar/tablas_composicion_alimentos/45-calidad.pdf
8. Di Rienzo, J. A. InfoStat versión 2020. UNC Argentina.

9. dos Santos, A. P. M.; Santos, E. M.; Silva de Oliveira, J.; Garcia Leal de Araújo, G.; de Moura Zanine, A.; Araújo Pinho, R. M.; Costa do Nascimento, T. V.; Fernandes Perazzo, A.; Ferreira, D. de J.; da Silva Macedo, A. J.; de Sousa Santos, F. N. 2023. PCR identification of lactic acid bacteria populations in corn silage inoculated with lyophilised or activated *Lactobacillus buchneri*. *Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza. Argentina.* 55(1): 115-125. DOI: <https://doi.org/10.48162/rev.39.101>
10. Faithfull, N. T. 2002. *Methods in Agricultural Chemical Analysis: A Practical Handbook.* CAB International. 304 p.
11. Huo, W.; Zhang, Y.; Zhang, L.; Shen, C.; Chen, L.; Liu, Q.; Guo, G. 2022. Effect of lactobacilli inoculation on protein and carbohydrate fractions, ensiling characteristics and bacterial community of alfalfa silage. *Frontiers in Microbiology.* 13: 1070175.
12. Krawutschke, M.; Thaysen, J.; Weiher, N.; Taube, F.; Gierus, M. 2013. Effects of inoculants and wilting on silage fermentation and nutritive characteristics of red clover grass mixtures. *Grass and Forage Science.* 68: 326-338. <https://doi.org/10.1111/j.1365-2494.2012.00892.x>
13. Kung, J. L.; Shaver, R. D.; Grant, R. J.; Schmidt, R. J. 2018. Silage review Interpretation of chemical, microbial, and organoleptic components of silages. *Journal of Dairy Science.* 101: 4020-4033. <https://doi.org/10.3168/jds.2017-13909>
14. Liu, Q.; Zong, C.; Dong, Z.; Wu, J.; Zhu, J.; Li, J.; Shao, T. 2020. Effects of cellulolytic lactic acid bacteria on the lignocellulose degradation, sugar profile and lactic acid fermentation of highmoisture alfalfa ensiled in low-temperature seasons. *Cellulose.* 27: 7955-7965. <https://doi.org/10.1007/s10570-020-03350-z>
15. Liu, Y. F.; Qiu, H. R.; Yu, X.; Sun, G. Q.; Ma, J.; Zhang, D. L.; Senbati, H. 2017. Effects of addition of lactic acid bacteria, glucose, and formic acid on the quality of *Amaranthus hypochondriacus* silage. *Acta Prataculturae Sinica.* 26: 214-220. DOI: 10.11686/cyxb2017164
16. Lotfi, S.; Rouzbehan, Y.; Fazaeli, H.; Feyzbakhsh, M. T.; Rezaei, J. 2022. The Nutritional Value and Yields of Amaranth (*Amaranthus hypochondriacus*) Cultivar Silages Compared to Silage from Corn (*Zea mays*) Harvested at the Milk Stage Grown in a Hot-humid Climate. *Animal Feed Science and Technology.* 289: 115336. <https://doi.org/10.1016/j.anifeedsci.2022.115336>
17. McDonald, P.; Henderson, A. R.; Heron, S. J. E. 1991. *The biochemistry of silage.* Chalcombe publications.
18. Mertens, D. R.; Grant, R. J. 2020. Digestibility and intake. *Forages: the science of grassland agriculture.* 2: 609-631. <https://doi.org/10.1002/9781119436669.ch34>
19. Miñon, D. P.; Gallego, J. J.; Barbarossa, R. A.; Margiotta, F. A.; Martinez, R. S.; Reinoso, L. 2009. Evaluación de la producción de forraje de híbridos de maíz para silaje en el Valle Inferior del Río Negro (campaña 2008-2009). INTA EEA Valle Inferior.
20. Mu, L.; Xie, Z.; Hu, L.; Chen, G.; Zhang, Z. 2021. *Lactobacillus plantarum* and molasses alter dynamic chemical composition, microbial community, and aerobic stability of mixed (amaranth and rice straw) silage. *Journal of the Science of Food and Agriculture.* 101: 5225-5235. <https://doi.org/10.1002/jsfa.11171>
21. Patterson, J. D.; Sahle, B.; Gordon, A. W.; Archer, J. E.; Yan, T.; Grant, N.; Ferris, C. P. 2021. Grass silage composition and nutritive value on Northern Ireland farms between 1998 and 2017. *Grass Forage Science.* 76: 300-308. <https://doi.org/10.1111/gfs.12534>
22. Pineda, J. A.; Sánchez, M. E.; Scaramuzza, J. P. 2015. Estudio comparativo de calidad y valor nutritivo de silos bolsa de maíz en la zona de James Craik-Córdoba (Bachelor's thesis).
23. Pšariková B.; Peterka, J.; Trcková, M.; Moudry, J.; Zralý, Z.; Herzig, I. 2007. The content of insoluble fibre and crude protein value of the aboveground biomass of *Amaranthus cruentus* and *A. hypochondriacus*. *Czech Journal of Animal Science.* 52: 348-353. DOI:10.17221/2339-CJAS
24. Queiroz, O. C. M.; Arriola, K. G.; Daniel, J. L. P.; Adesogan, A. T. 2013. Effects of 8 chemical and bacterial additives on the quality of corn silage. *Journal of Dairy Science.* 96(9): 5836-5843.
25. Rahjerdi, N. K.; Rouzbehan, Y.; Fazaeli, H.; Rezaei, J. 2015. Chemical composition, fermentation characteristics, digestibility, and degradability of silages from two amaranth varieties (Kharkovskiy and Sem), corn, and an amaranth-corn combination. *Journal of Animal Science.* 93: 5781-5790. <https://doi.org/10.2527/jas.2015-9494>
26. Ramírez Ordóñez, S.; Rueda, J. A.; Medel Contreras, C. I.; Hernández Bautista, J.; Corral Luna, A.; Portillo, M. F. 2023. Effect of cutting height, a bacterial inoculant and a fibrolytic enzyme on corn (*Zea mays* L.) silage quality. *Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza. Argentina.* 55(2): 129-140. DOI: <https://doi.org/10.48162/rev.39.115>
27. Reinoso, L. G.; Martinez, R. S.; Otegui, M. E.; Mercau, J.; Gutierrez, M. 2018. Rendimiento potencial de maíz en los valles de Norpatagonia: una aproximación desde los modelos de simulación.
28. Rezaei, J.; Rouzbehan, Y.; Fazaeli, H. 2009. Nutritive value of fresh and ensiled amaranth (*Amaranthus hypochondriacus*) treated with different levels of molasses. *Animal Feed Science and Technology.* 151: 153-160. <https://doi.org/10.1016/j.anifeedsci.2008.12.001>
29. Rezaei, J.; Rouzbehan, Y.; Fazaeli, H.; Zahedifar, M. 2014. Effects of substituting amaranth silage for corn silage on intake, growth performance, diet digestibility, microbial protein, nitrogen retention and ruminal fermentation in fattening lambs. *Animal Feed Science and Technology.* 192: 29-38. <https://doi.org/10.1016/j.anifeedsci.2014.03.005>

30. Rohweder, D.; Barnes, R. F.; Jorgensen, N. 1978. Proposed hay grading standards based on laboratory analyses for evaluating quality. *Journal of Animal Science*. 47: 747-759. <https://doi.org/10.2527/jas1978.473747x>
31. Seguin, P.; Mustafa, A. F.; Donnelly, D. J.; Gélinas, B. 2013. Chemical composition and ruminal nutrient degradability of fresh and ensiled amaranth forage. *Journal of the Science of Food and Agriculture*. 93(15): 3730-3736.
32. Silveira Pimentel, P. R.; dos Santos Brant, L. M.; Vasconcelos de Oliveira Lima, A. G.; Costa Cotrim, D.; Costa Nascimento, T. V.; Lopes Oliveira, R. 2022. How can nutritional additives modify ruminant nutrition? *Revista de la Facultad de Ciencias Agrarias*. Universidad Nacional de Cuyo. Mendoza. Argentina. 54(1): 175-189. DOI: <https://doi.org/10.48162/rev.39.076>
33. Slottner, D.; Bertilsson, J. 2006. Effect of ensiling technology on protein degradation during ensilage. *Animal feed science and technology*. 127(1-2): 101-111.
34. Tamminga, S.; Ketelaar, R.; Van Vuuren, A. M. 1991. Degradation of nitrogenous compounds in conserved forages in the rumen of dairy cows. *Grass and Forage Science*. 46(4): 427-435.
35. Verbič, J.; Ørskov, E. R.; Žgajnar, J.; Chen, X. B.; Žnidaršič-Pongrac, V. 1999. The effect of method of forage preservation on the protein degradability and microbial protein synthesis in the rumen. *Animal feed science and technology*. 82(3-4): 195-212.
36. Villa, R.; Rodríguez, L. O.; Fenech, C.; Anika, O. C. 2020. Ensiling for anaerobic digestion: A review of key considerations to maximise methane yields. *Renewable and Sustainable Energy Reviews*. 134: 110401. <https://doi.org/10.1016/j.rser.2020.110401>
37. Wan, J. C.; Xie, K. Y.; Wang, Y. X.; Liu, L.; Yu, Z.; Wang, B. 2021. Effects of wilting and additives on the ensiling quality and *in vitro* rumen fermentation characteristics of sudangrass silage. *Animal Bioscience*. 34(1): 56. DOI: 10.5713/ajas.20.0079
38. Zhou, Y.; Drouin, P.; Lafrenière, C. 2019. Effects on microbial diversity of fermentation temperature (10°C and 20°C), long-term storage at 5°C, and subsequent warming of corn silage. *Asian-Australasian Journal of Animal Sciences*. 32: 1528. DOI:10.5713/ajas.18.0792
39. Zubillaga, M. F.; Camina, R.; Orioli, G. A.; Barrio, D. A. 2019. Response of *Amaranthus cruentus* cv Mexicano to nitrogen fertilization under irrigation in the temperate, semiarid climate of North Patagonia, Argentina. *Journal of plant nutrition*. 42: 99-110. <https://doi.org/10.1080/01904167.2018.1549674>
40. Zubillaga, M. F.; Camina, R.; Orioli, G.; Failla, M.; Barrio, D. A. 2020. Amaranth in southernmost latitudes: plant density under irrigation in Patagonia, Argentina. *Revista Ceres*. 67: 93-99. <https://doi.org/10.1590/0034-737X202067020001>
41. Zubillaga, M. F.; Martínez, R. S.; Camina, R.; Orioli, G. A.; Failla, M.; Alder, M.; Barrio, D. A. 2021. Amaranth irrigation frequency in northeast Patagonia, Argentina. *Biotechnologie, Agronomie, Société et Environnement*. 25: 247-258. DOI: 10.25518/1780-4507.19310

ACKNOWLEDGEMENTS, FINANCIAL SUPPORT AND FULL DISCLOSURE

Financial support from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad Nacional de Río Negro is gratefully acknowledged. The authors thank to INTA Valle Inferior del Río Negro for the possibility to conduct this study.

Authors assures there is no actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations.