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WILLOW LEAF SILAGE: EVALUATION OF QUALITY, ORGANOLEPTIC CHARACTERISTICS, ACCEPTABILITY, AND RUMEN DEGRADABILITY IN GOAT FEED

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ABSTRACT

This study aims to evaluate the nutritional composition, pH evolution, and organoleptic characteristics, acceptability, and rumen degradability of willow leaf silage intended for goat feeding. Leaves and edible stems of "Ragonese 131-27 INTA" willow (*Salix babylonica* x *Salix alba*) were collected, chopped and then ensiled for 84 days by chipping. Three different microsilages were evaluated: Willow leaf silage, willow leaf silage with 20% corn grain, and willow leaf silage with 20% wheat bran. The pH was measured, and a pH evolution curve was constructed for the different microsilages. The nutritional composition was evaluated in terms of dry matter, crude protein, neutral detergent fibre, acid detergent fibre, and organic matter. The organoleptic characteristics and acceptability test in goats were also determined. The willow leaf silage with 20% wheat bran recorded the best conservation and nutritional composition, and a better acceptance by goats with respect to the rest of the silages. Regarding rumen degradability, the willow leaf silage with 20% corn grain had the highest performance. Therefore, willow leaf silage constitutes a recommendable alternative feed for goats.

Key words: Alternative feed, goats, silage, willow, *Salix* spp.

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INTRODUCTION

The intensification of animal production and the occurrence of droughts due to climate change have led to a greater dependence on external supplies of concentrated feed and conserved fodder, which are generally expensive. This, along with price volatility and the progressive deterioration of the productive capacity due to soil degradation, represents the most significant threat to the viability of the sector (Sivilaiand Preston, 2019; Arias et al., 2020). In this context, there is the need for new feeding alternatives for production, which would allow independence from external resources and an optimal productive response at a lower cost and in an environmentally friendly way (Sivilaiand Preston, 2019; Arias et al., 2020).

In Argentina, the Salado basin is an agricultural region with a predominance of livestock farming, where forestation with Salicaceae (willows and poplars) has great potential as a complementary production, providing shade and protection for livestock and wood for installations, attenuation of excess water, raw material for industry and carbon dioxide fixation, among other qualities (Rossi and Torrá 2006; Carou et al., 2010; Casaubón et al., 2017). In most forest species in silvopastoral systems, foliage is known to be palatable and contain nutrients suitable for livestock feeding (Rossi and Torrá 2006; Carou et al., 2010; Casaubón et al., 2017). In this sense, foliage from pruning, seasonally fallen leaves, browsed or ensilaged leaves obtained from forestry activities would be a nutritional complement to the pastures present in the system (Rossi and Torrá 2006; Carou et al., 2010; Casaubón et al., 2017), although seasonality and deciduous behaviour of the species must be considered. Poplar and willow leaves have been shown to efficiently supplement goat, sheep, and cattle (Rossi and Torrá, 2006; Muklada et al., 2020; Awabdeh et al., 2022). Different studies have proved that willow leaves are a valuable forage for livestock. In terms of composition and forage value of the leaves, the literature has reported average crude protein (CP) values of 19% and 29.47% in spring (Rossi and Torrá 2006; Carou et al., 2010; Casaubón et al., 2017). Furthermore, it has been determined that willow leaves have an anthelmintic effect, with ovicidal and larvicidal activity *in vitro* in the control of *Haemonchus contortus* (Schapiro et al., 2016), a very important nematode in production due to its adverse effects on small ruminants (Steffen et al., 2022).

It is necessary to deepen the knowledge of the capacity of locally available leaves and agricultural and forest raw materials to provide nutrients, and thus determine if they constitute a feed option for livestock. It should be noted that in Argentina there is little information on the nutritional composition and forage value of willow leaves. Furthermore, there are no reports on the form of storage (silage) given the deciduous behaviour of its leaves, nor on its acceptance by goats. Therefore, this work aims to evaluate the nutritional composition, pH evolution, organoleptic characteristics, acceptability and rumen degradability of the dry matter and fibre fraction of willow leaf silage intended for goat feeding.

MATERIALS AND METHODS

Location and duration

The study was carried out at the Caprine Teaching Unit (-34.911357, -57.927385) of the Faculty of Agriculture and Forestry Sciences, National University of La Plata, Buenos Aires, Argentina. The procedures developed on the animals were approved by the Institutional Committee for the Care and Use of Laboratory Animals (CICUAL) of the School of Veterinary Sciences of the National University of La Plata (protocol 62-6-17 T and 97-1-19T).

Silage preparation

Briefly, leaves and edible stems (<5 mm diameter) were collected randomly during the autumn season from several young and mature trees of "Ragonese 131-27 INTA (Instituto Nacional de Tecnología Agropecuaria) willow (*Salix babylonica* x *Salix alba*), freshly chopped at a length of 2 to 3 cm, and immediately ensiled. The silage was made in 5-litre PVC buckets (0.20 m high x 0.21 m in diameter), with airtight lids and holes in the base for the evacuation of liquids. Black 6-gauge polyethylene plastic bags were inserted twice. The microsilages were filled manually in layers, and the shredded fine stems and leaves were tramped down to ensure adequate compaction and air elimination to guarantee an adequate fermentation process. Once the filling process was completed, the plastic bags were hermetically closed with plastic clamps expelling all the air, the microsilages were labelled on the lid and body of the bucket and placed out of reach of rodents and at a temperature of 20-29°C (Fig. 1). Three different microsilages were made:

1) Willow Leaf Silage with 20% corn grain (WLSCG)
2) Willow leaf silage with 20% wheat bran (WLSWB)
3) Willow Leaf Silage (WLS)

Evaluation of microsilages

Microsilages were opened at 21, 42, 63 and 84 days after processing for evaluation.

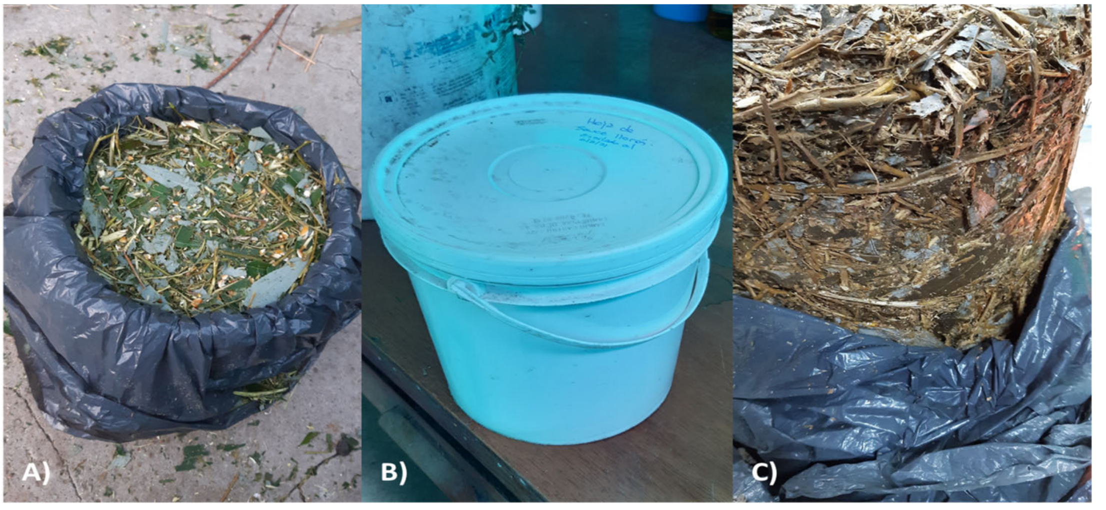

Fig. 1. Microsilage manufacturing process. Reference: A) Preparation of willow leaf silage with 20% corn grain; B) Microsilages hermetically sealed, labelled, and ready for storage; C) Observation of microsilages after 84 days of fermentation.

pH measurement

Ten grams (10 g) of silage were taken and added to 50 mL of distilled water for 15 minutes to release the fermentable juices into the aqueous medium. The solution was then measured with a pH meter, and a pH evolution curve was constructed for the different microsilages.

Subsamples were taken on each sampling day (10 g/sample) and then frozen until analysis. A pool was made with the subsamples, and the following nutritional parameters were evaluated: dry matter (DM), organic matter (OM), neutral detergent fibre (NDF), acid detergent fibre (ADF), and crude protein.

Dry matter (DM)

50-g samples of the different silages were oven dried (SOMCICbrand) at 95-100°C for 24 h or until constant weight. The dry matter content was calculated by weight difference according to the following equation:

DM (%) = 100*((Wi-Wf) /Wi)

where Wi is the initial weight of the sample and Wf is the final weight. Determinations were carried out in duplicate for each microsilage.

Organic matter (OM)

The first step in determining organic matter is to analyse the ash (AOAC, 2002). Ash is most performed by burning the sample at 550-600°C in a muffle furnace. Organic matter is that portion of the feed that is not water or ash (i.e., C, H, O, N bonding).

% organic matter = 100 - Ash

Neutral detergent fibre (NDF) and acid detergent fibre (ADF)

For the determination of NDF and ADF, the Van Soest technique modified by Komarek (1994) was followed. Thermostable α-amylase (Sigma, A3306) and sodium sulphite were used. Determinations were carried out in duplicate for each microsilage.

Crude protein (CP)

Total nitrogen was determined according to the Kjeldahl-N method (AOAC, 1995). The percentage of CP was calculated by affecting the N value obtained by the correction factor 6.25. Determinations were carried out in duplicate for each microsilage.

Organoleptic characteristics

The organoleptic characteristics were evaluated at the time of opening the silages, using the silage evaluation sheets proposed by Chaverra and Bernal (2000) for: Colour (Green-brownish; Olive green; Dark green), Odour (Vinegary; Sweet fruity; Acid fruity), Texture (Excellent; Good; Regular; Poor) and Humidity (Excellent; Good; Regular; Poor), applying the characteristics assessed in standardised ranges of: Excellent, Good, Regular and Poor.

Degree of preference or acceptability

Five goats were fed 150 g of each silage, which was supplied simultaneously in three feeders, and the ingestive behaviour of preferential choice was determined. Each silage was classified into grade 1, 2 and 3 according to the degree of priority of acceptance and ingestion within the first 30 minutes.

Evaluation of rumen degradability of silage

In situ rumen degradability was determined as previously described by Arias et al. (2020). Briefly, Ankom brand polyester bags of 10 x 10 cm in size, with a pore size of 40 to 60 microns, were used. Ten mg/cm^2 of sample was placed inside each bag in duplicate: fresh willow leaf (T1), willow leaf silage (T2), willow leaf silage with 20% corn (T3), and willow leaf silage with 20% wheat bran (T4). The samples were incubated for 48 h, then the material was removed from the rumen to be washed for periods of 10 minutes, until the fluid was clear and dried in an oven at 90-95ºC for 24 h (AOAC, 1995; Arias et al., 2020). Dry matter degradability was determined from the difference in sample weight before and after in situ incubation in the nylon bags. After incubation, NDF and ADF were determined, and the degradability coefficients of these fractions were calculated.

Design and statistical analysis

The data were analyzed using the mixed procedure (SAS, 2004) for a completely randomized block experimental design, using a model that included the fixed effect of sampling (treatment) and the random effect of the animal. To evaluate the effect of time on the evolution of the pH of the different microsilages, an analysis of repeated units was carried out. Data were analyzed using ANOVA, and statistical differences were determined by Tukey's test. Differences were considered significant with a p value <0.05.

RESULTS AND DISCUSSION

The results found in relation to the nutritional quality for fresh willow leaf without silage (FWL) were 34.0% DM and 11.1% CP, being below the values reported in previous studies in Argentina (Rossi and Torrá, 2006; Carou et al., 2010; Casaubon et al., 2017), in New Zealand (McWilliam et al., 2005), and Israel (Muklada et al., 2018; 2020; 2021). Table 1 shows the chemical composition of the different microsilages. The significantly lowest values of DM (35.8%) were observed in the WLS treatment, along with the highest values of NDF (57.2%). WLSWB showed the highest CP values and intermediate NDF values compared to WLS and WLSCG. The lowest NDF and CP values were obtained in WLSCG. The low values in WLSCG could be because maize grain has low NDF and CP contents (14.3 and 7.8%, respectively). In relation to the composition, forage with less than 50% NDF and more than 15% CP is regarded as a good quality source, while forage with less than 50% DM, greater than 65% NDF and less than 8% CP is regarded as a low quality source (Di Marco, 2011). Accordingly, WLS has a medium to low quality, while WLSCG and WLSWB presented a higher quality due to the incorporation of corn and wheat bran. This agrees with Muklada et al. (2021), who found that willow forage can be successfully ensiled without affecting the original quality of the green forage. In addition, it has been determined that willow silage may constitute 20% of the DM in weaned lambs, 28 and 25.6% of the total DM of willow forage consumed by goats (Muklada et al., 2018; Muklada et al., 2020; Awabdeh et al., 2022). However, a study conducted by Casaubón et al. (2017) revealed the forage quality in all the microsilages showed higher values than those obtained in the natural pasture of the region (9.8%). The climatic conditions of the production areas, variety and crop management, and the season in which the samples were taken for analysis must be considered, because they directly influence the original composition of the forage (Chaverra and Bernal, 2000; Larsen et al., 2020; Muklada et al., 2021). Given its deciduous characteristics (the leaves fall in winter) and the critical lack of forage at this time of year, collection, storage and ensilage are required to

Table 1. Chemical composition of willow leaf microsilages.

Microsilages	DM (%)	OM (%)	NDF (%)	ADF (%)	CP (%)
Willow Leaf Silage	35.8	93.3	57.2	33.9	11.4
Willow Leaf Silage with corn grain	39.9	95.2	39.22	25.5	9.5
Willow Leaf Silage with wheat bran	38.7	92.9	46.6	20.6	12.6

DM: dry matter expressed in %; OM: organic matter expressed in %; NDF: neutral detergent fibre expressed in %; ADF: acid detergent fibre expressed in %; CP: crude protein expressed in %.

optimise its use before winter. This will ensure that forage retains its original nutritional value. Forage can be preserved over time, ensuring availability of green leaves and tender branches with better palatability (Chaverra and Bernal, 2000).

There are several important factors that determine the quality of silage. In this sense, pH reference values around 4.2 – 4.4 determine a favourable environment for the correct process, inhibiting pathogen growth and preserving the nutritional characteristics of the ensiled product (Chaverra and Bernal, 2000; Larsen et al., 2020; Muklada et al., 2021). In the present study, the pH values ranged between 4.74 and 4.86, being higher in WLS and lower in WLSWB. These findings agree with those of Larsen et al. (2020) and Muklada et al. (2021), who found similar pH values of 5.5 to 4.5 when harvested in autumn (September to October), and 5.10 in control silages without additives, respectively. However, Muklada et al. (2021) managed to reduce the pH to 4.4 by inoculating with lactic acid bacteria. Regarding the average pH values of the different microsilages in the present study, even without statistical differences ($p>0.05$), the smallest numerical differences were recorded in WLSWB. The remains of floury endosperm of the by-product, in addition to the fine degree of grinding, would possibly allow better compaction in the ensiling process, achieving a more favourable environment for the development of lactic bacteria responsible for the drop in pH (Fig. 2).

Regarding the analysis of repeated units on the effect of time on the evolution of pH in all microsilages, the lowest value ($p<0.05$) was recorded on day 42 (Fig. 3). From that day on, it can be considered that the pH values stabilized. The following records were higher, probably due to successive openings for the corresponding measurements. In this sense, several authors have mentioned that the planning of daily consumption is very important to avoid making unnecessary openings that could cause deterioration in the quality of the silage (Chaverra and Bernal, 2000; Larsen et al., 2020; Muklada et al., 2021).

The analysis of the organoleptic characteristics observed for all microsilages coincides with the optimal global indicators for ensiled materials determined by Chaverra and Bernal (2000), presenting correct fermentative and nutritional values. The microsilages that incorporated corn (WLSCG) or wheat bran (WLSWB) presented a fruity aroma. The distinctive odours found in different microsilages, and the varying fermentation times are a clear indication of the decrease in pH caused by the proliferation of lactic acid bacteria. This gives the silage a pleasant smell and prevents the formation of sufficient quantities of butyric acid, increasing the pH and consequently resulting in unpleasant odours with total or partial loss of silage. (Chaverra and Bernal, 2000). The advantage of good smell is related to the preference and greater consumption by animals. The appearance and colour of silage determine the characteristics

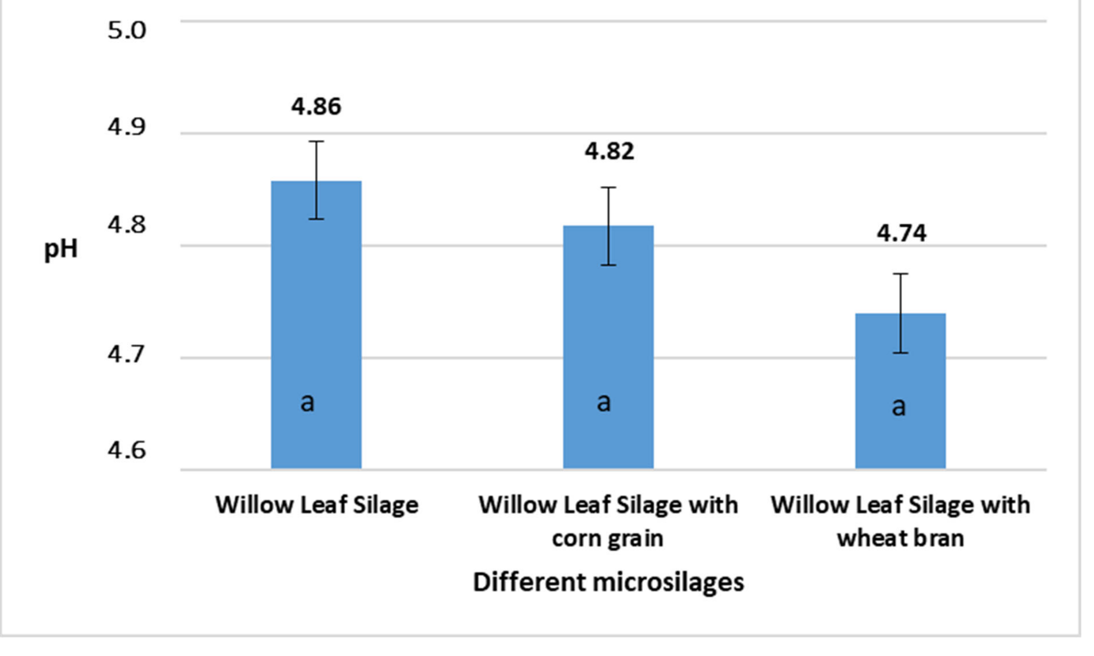

Fig. 2. pH average values of the three different microsilages in the study period.

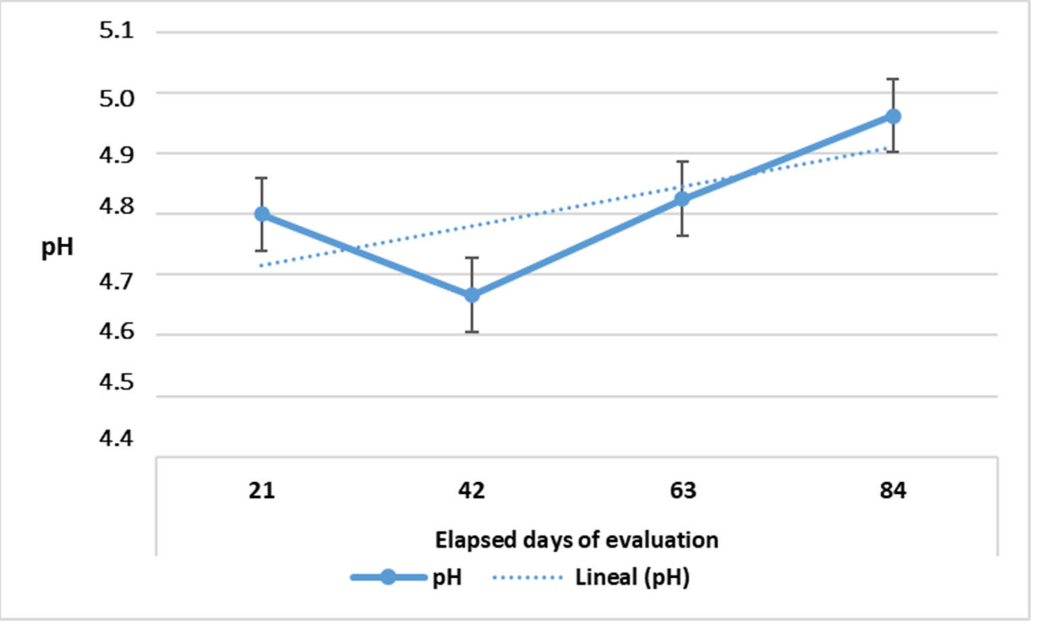

Fig. 3. Effect of time on the average pH of the different microsilages during the study period.

of the ensiling process, after proper anaerobic fermentation (Chaverra and Bernal, 2000). In this regard, the microsilages with willow leaves and 20% corn presented an excellent colour, similar to the original colour of the willow before ensiling; whereas the addition of 20% bran presented a slightly darker green colour classified as regular (Fig. 1C). The best texture (excellent-compact) was recorded in WLSWB, showing that the incorporation of additives with grains favours the reduction of humidity and preservation of the silage. Conversely, WLSCG had the highest degree of disintegration, which could be due to the grain grinding degree (Chaverra and Bernal, 2000). Humidity varied from good to fair in the microsilages (Table 2).

The acceptability test confirmed that all goats used (n=5) first accepted WLSWB. Secondly, four accepted WLSCG and only one WLS; and thirdly, four accepted WLS and only one WLSCG (Table 3). Regardless of the degree of acceptance (1st, 2nd, and 3rd), the 150-g silage samples placed on each tray were completely consumed, indicating that willow silage (with or without additives) could be a good alternative feed for goats.

There are studies that show that phenolic components in willow extracts have anti-inflammatory, anti-rheumatic, antipyretic, antidote, and antiseptic properties (Van Wyk and Wink, 2017). The consumption of willow leaves produces certain biological effects on animals, improving their health, acting through the attenuation of gastrointestinal parasitosis (Schapiro et al., 2016), and optimising the protein transit to the duodenum (Rossi and Torrá, 2006). In the present study, phenolic compounds determination was not carried out, remaining as a topic for future research.

With respect to rumen degradability of dry matter, after 48 h of incubation, T4 was significantly higher ($p<0.05$) than the rest of the treatments. T1 and T3 had similar behaviours. T2 was the treatment with the lowest rumen degradability ($p<0.05$). Regarding rumen degradability of NDF, it was verified that T3 was significantly greater than greater ($p<0.05$) than the rest of the treatments, while T4 exhibited a significantly lower ($p<0.05$) rumen degradability. Similar degradability was observed in T1 and T2 ($p>0.05$). According to Fino et al. (2013), the rumen degradability of willow (*S. humboldtiana* Willd.) dry matter was high at 48 h (71.05%). However, a study conducted by Hathaway (1985) reported lower values of 67.6., 47.6 and 53.7% at 48 h for *S. matsudana*, being more in line with the results obtained in the present study. At 48 h of incubation, Fino et al. (2013) reported rumen degradability values of the willow NDF fraction of 67.43%. Similar values were observed in the treatment that included maize in the silage, but in the rest of the silages the degradability was lower. With respect to the rumen degradability

Table 2. Organoleptic characteristics of the different willow leaf microsilages.

	Organoleptic indicators for silage evaluation			
Microsilages	**Colour**	**Odour**	**Texture**	**Humidity**
Willow Leaf Silage	Green-brownish	Regular (Vinegary)	Good	Good
Willow Leaf Silage with corn grain	Olive green	Slightly pleasant (Sweet fruity)	Regular (disintegrated)	Good
Willow Leaf Silage with wheat bran	Slightly dark green	Pleasant (Acid fruity)	Excellent (compact)	Regular

Table 3. Analysis of the acceptability test of the different microsilages.

Goat assessed	Acceptability grade 1	Acceptability grade 2	Acceptability grade 3
Goat 1	Willow Leaf Silage with wheat bran	Willow Leaf Silage	Willow Leaf Silage with corn grain
Goat 2	Willow Leaf Silage with wheat bran	Willow Leaf Silage with corn grain	Willow Leaf Silage
Goat 3	Willow Leaf Silage with wheat bran	Willow Leaf Silage with corn grain	Willow Leaf Silage
Goat 4	Willow Leaf Silage with wheat bran	Willow Leaf Silage with corn grain	Willow Leaf Silage
Goat 5	Willow Leaf Silage with wheat bran	Willow Leaf Silage with corn grain	Willow Leaf Silage

of ADF, T2 and T3 had similar and significantly higher ($p<0.05$) degradability values than T1 and T4, but these two treatments did not differ significantly ($p>0.05$) from each other (Fig. 4). The greatest degradability of the rumen fibre was observed in T3, probably due to a higher energy concentration by the protein in relation to T1, T2 and T4 (0.26 vs 0.12, 0.20 and 0.21, respectively). Likewise, T3 presented low NDF values (39.2%). When carbohydrates are in the form of starches, they have little participation in the ensiling process, because amylolytic bacterial enzymes such as those developed during ensiling have low activity in acidic environments (Neureiter et al., 2005). Yitbarek and Tamir (2014) used sources rich in starches as additives for forage conservation and found that between 90-100% of the starch in barley and oats was recovered after ensiling. This has an important implication for goat production, given the adaptability of goats to harsh and hostile rearing environments, coupled with the scarcity of planning, technology, input costs, and the access to water for this production. Therefore, these alternative feeds can become an accessible and sustainable resource to improve animal production. Willow silage can be available year-round and can be compatible with off-season goat production in any region, being cheap and easy for smallholder families to apply in order to develop healthy, productive, and profitable goat herds.

CONCLUSIONS

This is the first report on willow silage as goat feed in Argentina. Willow leaf has good nutritional qualities for conservation through the silage method that make it an extremely suitable alternative material for feeding goats. Of the combinations evaluated, willow leaf silage with 20% wheat bran (WLSWB) resulted in better conservation and nutritional composition. However, the willow leaf silage with 20% corn grain (WLSCG) demonstrated the greatest rumen degradability of the fibre fraction.

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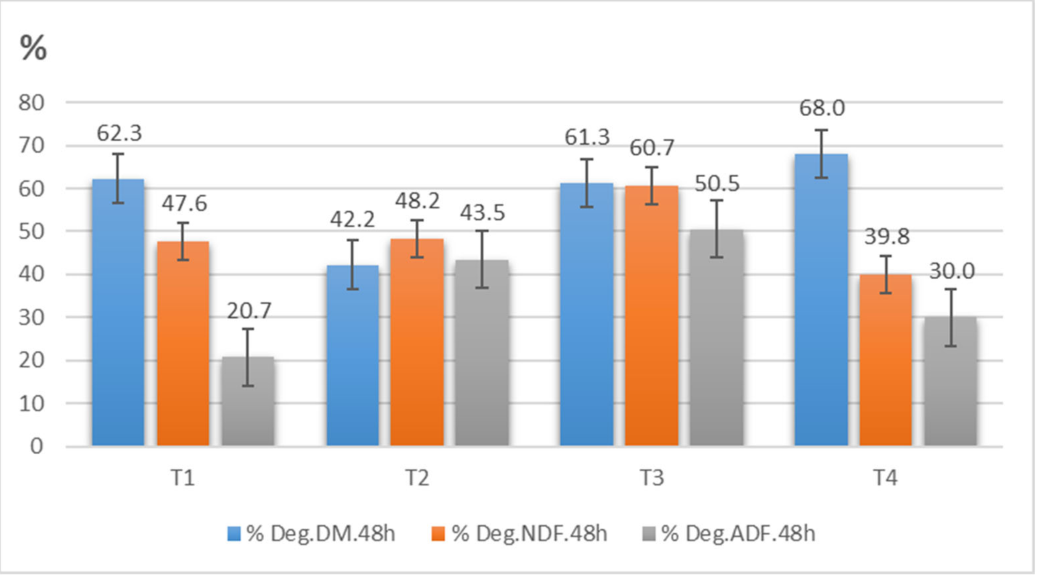

Fig. 4. Rumen degradability of post-incubation dry matter (DM), neutral detergent fibre (NDF), acid detergent fibre (ADF) at 48 h according to treatments. Reference: % Deg.DM48h: Rumen degradability of post-incubation DM at 48 h, expressed as a percentage; % Deg.NDF48h: Rumen degradability of post-incubation NDF at 48 h, expressed as a percentage. % Deg. ADF48h: Rumen degradability of post-incubation ADF at 48 h, expressed as a percentage.

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Author contributions

All authors participated in the bibliographic review and contributed to the conceptualisation and design of the study. The methodology, data collection, analysis and discussion of results were developed by Kevin Steffen, Rubén Arias, Soledad Trigo, and Lucas Rodrigues. The initial draft of the manuscript was prepared by Kevin Steffen and Rubén Arias, and all authors provided feedback on previous versions. Gabriela Muro and Ángel Cordiviola supervised the study. All authors revised the final version of the manuscript.

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