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Physicochemical and Preference Evaluation of Silages from *Cucurbita argyrosperma* Huber Residues and Its Effect on the Production and Composition of Milk from Dual-purpose Cows in Campeche, Mexico: Pilot Study

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Abstract: Livestock systems can contribute to food security by making use of available resources not suitable for human consumption such as *Cucurbita argyrosperma* Huber residue (CR). The aim of this pilot study was to evaluate preference and physicochemical characteristics of CR silages and their effect on milk production and milk composition from cows in a dual-purpose system in Campeche, Mexico. Three completely randomized experiments were performed. In experiment 1, physicochemical characteristics of four CR plus hay grass silages were evaluated: CR-77, CR-71, CR-65 and CR-59, containing 77%, 71%, 65% and 59% of CR, with 25%, 30%, 35% and 40% of estimated dry matter (DM), respectively. In experiment 2, silages intake preferences were determined in cattle. In experiment 3, the effect of CR silage or corn silage on milk production and milk composition of grazing cows in a dual-purpose system was evaluated. The results in experiment 1 showed that DM content differed between treatments ($p < 0.05$), ranging from 27.35% (CR-77) to 41.81% (CR-59) and estimated DM was similar to the actual values. pH and temperature were also different ($p < 0.05$). pH was different between CR-71 (4.01) and CR-59 (4.43), and temperature between CR-77 (28.87 °C) and CR-65 (29.5 °C). Crude protein (CP) was very low in all silages ($<4\%$), particularly in CR-59 (2.8%) ($p < 0.05$), and neutral detergent fiber (NDF) was significantly ($p < 0.05$) higher with CR-65 and CR-59 (average: 79.6%) than CR-77 and CR-71 (average 75.1%). There were no differences in intake preference (experiment 2), milk production (average 3.7 L) or milk composition (average protein: 3.27%, fat: 2.26%, lactose: 5.23%, solids non-fat: 9.17%) (experiment 3) ($p > 0.05$), but differences in DM intake of silages in experiment 3 were observed ($p < 0.05$). Even with the limitations of this pilot study, it is feasible to produce silages with CR with a good fermentation process as a feeding alternative for cows in a dual-purpose system in Campeche, Mexico.

Keywords: pumpkin; cattle; vegetable residue; ruminant nutrition; new feedstuffs

1. Introduction

Grazing is considered the cheapest alternative to feed livestock. Fresh forage is a source of nutrients [1], but in most of the tropical areas of Mexico, it is only available during the rainy season. Another drawback of grazing is the constant decrease in land availability for cultivation of forages and pastures [2] due to the degradation of land for overgrazing (caused by extensive grazing systems based on natural grasslands in marginal environments) or to the change in its intended use [3]. However, the demand for food of animal origin is increasing due to the increase in the human population [4]. It is estimated that by 2050, the world population will be 9.7 billion people, while by 2100 it will be 11.2 billion [5]. Therefore, livestock systems have the challenge of increasing production levels with the least environmental impact, while being economically viable and socially responsible [6] so they contribute in a sustainable way to food safety [7].

An alternative to solve the lack of feed for livestock is the use of agricultural residues, which have the limitation of having a high cellulose content and a low nutritional value, which do not cover the maintenance requirements of animals [2]. Another option is the incorporation of unconventional foods, not suitable for human consumption, that reduce feed–food competition [8,9] with plant residues being an alternative for such an end. Plant residues contain carbohydrates, vitamins, minerals, antioxidants and fatty acids [9], and can be used as part of the cattle diet, replacing other ingredients, without affecting weight gain or milk production [10–12].

In Mexico, the pumpkin known as “chihua” (*Cucurbita argyrosperma* Huber) is mainly cultivated in the southern region of Mexico [13] and is sown on an area of 58,949.11 ha, for seed production [14,15], however, the rest of the pumpkin (peel and pulp) is discarded [13]. In a recent review, Valdez-Arjona and Ramírez-Mella [16] mention that pumpkin residue (*Cucurbita* sp.) can be a source of food for livestock, not only because of its nutritional value, but also due to its antioxidants, vitamins, minerals and pigments content that can improve both the quality of food of animal origin and animal health. Nonetheless information regarding the use of *Cucurbita argyrosperma* Huber in animal feed is still scarce. However, in an in vitro study it was shown that the dry residue of this pumpkin improves the degradability of dry matter (DM) in diets with high forage content [17].

One of the disadvantages of growing *Cucurbita argyrosperma* Huber is its seasonality since it is generally harvested once a year. In addition, it has a high moisture content (>90%) [13], a common feature in many plant residues that favors rapid decomposition, which makes the use of some form of conservation essential [18]. Silage is a conservation method that allows forage to be offered during the time of least availability of pasture and to maintain constant production levels throughout the year [19]. Successful silages from various fruits and vegetables, such as cassava, beets, carrots, broccoli, squash, citrus, banana, and pineapple have been reported [20–22]. Therefore, the aim of this pilot study was to evaluate physicochemical characteristics and intake preference of silages made from residue of *Cucurbita argyrosperma* Huber, as well as their effect on the production and composition of milk cow from a dual-purpose system in the state of Campeche, Mexico.

2. Materials and Methods

All experimental procedures involving animals were according to the Official Mexican Standard (NOM-062-ZOO-1999) [23] which has the technical specifications for the production, care and use of laboratory animals. To achieve the objective of this investigation, three experiments were performed.

2.1. Experiment 1. Physicochemical Evaluation of Silages with Different Levels of *Cucurbita Argyrosperma* Huber Residue

Collection of the material. In September 2017, around 400 kg of CR (peel and pulp, mainly) were collected directly from the cultivation fields of the town of Santo Domingo Kesté, Campeche (latitude 19°49′8.61″N, longitude 90°51′7.77″W). Likewise, around 300 kg of hay from *Echinochloa polystachya* and *Brachiaria brizantha* were purchased from Champotón, Campeche (latitude N19°21′1.51″, longitude 90°42′35.68″) [24].

Preparation of the silages. The collected material (CR and grass hay) was transferred to the Colegio de Postgraduados campus Campeche (latitude 19°50'13.8"9, longitude 90°58'61.1"1) [24] for the preparation of the silages. The CR was washed with water to remove dirt and debris. Subsequently, both the CR and the hay were chopped, and an inoculant made with molasses (56%), natural yogurt (37%) and mineral salts (6%) was added. Once a homogeneous mixture was obtained, 25-kg silos were made using 600-gauge black polyethylene bags and a manual silage machine to compact the mixture and remove most of the oxygen possible. Finally, the bags were sealed with plastic ties and industrial adhesive tape and stored indoors for 45 days at room temperature (28 °C). For more details, please refer to Supplementary Material S1.

Treatments. Four treatments were evaluated, which had different CR content (fresh basis): CR-77, CR-71, CR-65 and CR-59, with 77%, 71%, 65% and 59% of CR, and an estimated DM content of 25%, 30%, 35% and 40%, respectively. As we used a non-commercial inoculum, no general guidelines are available; thus we decided to use 3% of inoculum in all treatments, allowing us to maintain uniformity during silage preparation. All treatments were performed in quadruplicate. Table 1 shows the content of the ingredients of each treatment.

Table 1. Ingredients (% fresh basis) and estimated dry matter (DM) content (%) of *Cucurbita argyrosperma* Huber residue (CR) silages used for physicochemical evaluation and preference intake in experiments 1 and 2.

Ingredients	Silages			
	CR-77	CR-71	CR-65	CR-59
CR	77	71	65	59
Grass hay	20	26	32	38
Inoculum	3	3	3	3
Estimated DM ¹	25.4	30.3	35.3	40.2

CR: *Cucurbita argyrosperma* Huber residue; DM: dry matter; ¹ estimated DM was calculated according to DM content: CR: 7% [13], grass hay: 89% (average Rye grass hay [25], and cool-season grass hay, all samples [26]) and inoculum: 74% (average sugarcane molasses [25], and molasses–sugarcane [26]).

Sample collection. About 1 kg of fresh material was collected from each silo. The collected material was placed in aluminum trays and dried at 55 °C in an oven until constant weight. Subsequently, the material was milled in a Willey type mill with a 1-mm sieve and stored in poly-paper bags kept at 25 °C, in a dry and dark place, until chemical analysis.

Physicochemical analysis. Physicochemical characteristics of the silages were evaluated as follows. Temperature: after 45 days, the silos were opened and the temperature of each one was immediately measured with a mercury thermometer at three reference points: one central and two between the central point and the bag, at a depth of about 20 cm. DM: it was determined after measuring the temperature. To do so, 100 g of silage was collected from each silo, placed in paper bags, and dried for 48 h in a forced air oven at a temperature of 80 °C. The sample was weighed afterwards. For the calculation of the DM content the following formula was used: $100 - ((\text{initial weight} - \text{final weight}) / \text{initial weight})$. pH: the pH was measured with a potentiometer. For this measurement, 10 g of silage was weighed and placed in beakers, 90 mL of distilled water was added and mixed with an electric stirrer for 5 min. Finally, the pH was measured before the material settled in the beaker. Chemical analysis: the content of ether extract (EE), ash and crude protein (CP) was determined according to the AOAC [27], while the content of neutral detergent fiber (NDF) and acid detergent fiber (ADF) was determined according to Van Soest et al. [28]. Volatile fatty acids (acetic, propionic and butyric acids): 10 g of silage was mixed with 50 mL of distilled water in an amber flask and after a minute of shaking, the contents were transferred to 50-mL conical tubes and centrifuged at 15,000 rpm for 5 min. Finally, 1 mL of supernatant was collected in a 2-mL microtube containing 1 mL of metaphosphoric acid at 25% and stored at −4 °C until gas chromatography analysis (Hewlett Packard® model 6890, San Diego, CA, USA) [29].

Experimental design and statistical analysis. A completely randomized design with four treatments and four repetitions was used. The results were analyzed with the GLM procedure of SAS version 9.0 [30], and the comparison of means was performed with the Tukey test ($p < 0.05$).

2.2. Experiment 2. Evaluation of the Intake Preference of Silages with Different Levels of Cucurbita Argyrospema Huber Residues in Cattle

Location. The experiment was carried out at Colegio de Postgraduados Campus Campeche, located in Sihochac, Champotón, Campeche (latitude 19°50′13.8″N, longitude 90°58′61.1″W) [24].

Animals and housing. Four non-castrated male steers from different crossbreeds of Sardo Negro, Gyr, Brahman and Pardo Suizo, with an average weight of 300 kg, approximately 15 months of age, and with no history of intake of CR or silage were used. The animals were housed in a 3 m × 10 m individual pens, with a cement and soil floor, metal tubes and galvanized sheets. The feeders were made of cement, 3 m long, 80 cm high, 60 cm wide and 25 cm deep, which were divided with wooden boards into 4 compartments (Supplementary Material S2). Before the experiment, the steers were dewormed with Doramectin (Doramic + ad3e[®], Laboratorio Microsules, Barros Blancos, Uruguay) and vaccinated against bovine paralytic rabies (Derri Plus[®] cepa P.V., Productora Nacional de Biológicos Veterinarios BIVE, Mexico City, Mexico) and Clostridium, Pasteurella, Mannheimia and Histophilus (Bacterina Biobac[®] 11 vías, BIOZOO, Zapopan, Mexico).

Basal diet and treatments. A TMR formulated with straw from *Echinochloa polystachya* and *Brachiaria brizantha* (65% DM); distillery dry grains (28% DM), ground corn (6.5% DM) and mineral premix (0.5% DM) were used as basal diet for the steers. The TMR composition was as follows: 2.2 Mcal/kg ME, 11% CP and 57% NDF, covering the requirement for beef cattle of 300 kg [31]. Daily, 10 kg of TMR was offered to each steer, divided in two schedules: 09:00 a.m. and 16:00 p.m., and water intake was ad libitum. The preparation of treatments and silages was the same as in experiment 1 (Table 1; Supplementary Material S1): CR-77, CR-71, CR-65 and CR-59.

Intake preference test. The steers were adapted to silages. During a 7-day period, 500 g DM of each of the silages was offered in the morning before the TMR. In the preference test, 2 kg DM of each CR silage was offered, separately but simultaneously, for 5 consecutive days (Supplementary Material S2). In order to avoid a bias, every day the location of the CR silages was rotated in the feeder (Table 2). The CR silages were offered at 8:00 a.m. for 20 min before the TMR was offered. Subsequently, non-consumed CR silages were removed from the feeder and weighed. Intake of each of the CR silages was calculated by difference using the following formula: intake, kg DM = offered silage, kg DM - refused silage, kg DM.

Table 2. Order of the CR silages offered to steers in the intake preference test (experiment 2).

Ítem	1	2	Day 3	4	5
Order in the feeder *	1, 2, 3, 4	2, 3, 4, 1	3, 4, 1, 2	4, 3, 2, 1	1, 2, 3, 4

* 1: CR-77; 2: CR-71; 3: CR-65; 4: CR-59.

Experimental design and statistical analysis. A completely randomized experimental design with four treatments and four repetitions was used. Statistical analysis was performed using the MIXED procedure of the SAS version 9.0 [30]. The model included treatment, location, day and treatment × location interaction. Steer was a random effect [32]. The comparison of means was made with the Tukey test ($p < 0.05$).

2.3. Experiment 3. Evaluation of *Cucurbita Arggyrosperma* Huber Silage in the Production and Composition of Cow's Milk

Location. The experiment was carried out at the Cabahuil farm, located at kilometer 205 of the Escárcega-Villahermosa federal highway, in Carmen, Campeche, Mexico (latitude 18°21'38.8"9, longitude 91°51'05.5"6) [24].

Silage preparation. CR silage: based on the results of experiments 1 and 2, the preparation of a silage with 77% CR and 23% hay of *Echinochloa polystachya* and *Brachiaria brizantha* was determined. In February 2019, around 900 kg of CR (peel and pulp, mainly) were collected directly from the cultivation fields of the town of Ruiz Cortínez, Campeche (latitude 19°47'5.83"3, longitude 90°35'8.33"3). Likewise, around 300 kg of hay of *Echinochloa polystachya* and *Brachiaria brizantha* pastures were purchased from Champotón, Campeche (latitude N19°21'1.51", longitude 90°42'35.68") [24]. The CR was washed with water to remove dirt and debris. Subsequently, CR and hay, were chopped and mixed. Once a homogeneous mixture was obtained, layers of the mixed material were placed in the silo by spraying the inoculant (BIOSILE®, Chr Hansen, Hørsholm, Denmark) according to the manufacturer's recommended dosages: 5 g of additive diluted in 4 L of water, per each ton of forage. Compaction was carried out with a tractor to eliminate spaces in the mixture and decrease the amount of air. The mixture was covered with polyethylene and soil to avoid contact with air (Supplementary Material S3). Corn silage: the corn silage was prepared with San Pablito Creole corn, commonly cultivated in Campeche, Mexico. Corn was harvested at 75 days, immediately chopped and placed in the silo while spraying the inoculant (BIOSILE®, Chr Hansen, Denmark). Compaction was carried out with a tractor. Afterwards it was covered with polyethylene and soil as with the CR silage. After 30 days of storage, both silos were opened and offered to cows.

Animals. All animals were vaccinated against bovine paralytic rabies (Derrievolt, Voltier®, Mexico city, Mexico) and against *Clostridium* and *Pasteurella* (Bacterina 8 vías Pier, Laboratorios Pier, Tehuacán, Mexico) and dewormed with ivermectin (Astrovectina 4% L.A., Laboratorios Astro Nova, Jalisco, Mexico) and albendazole (Alban 10%, Laboratorios Andoci, Mexico city, Mexico) prior to the experiment, according to the established protocol in the ranch. In total, 20 cows from different crossbreeds of Brahman, Brown Swiss, Holstein, Sardo Negro and Gyr, 8 to 10 years old, with a calf less than 5 months old and an average milk yield of 3.8 ± 1.7 L d⁻¹ and body condition score of 1.5 ± 0.4 were used. Cows were randomly assigned in 2 groups of 10 animals each. The cows from Cabahuil farm belong to a double purpose system, which is representative of the cattle system production in the tropical regions of Mexico, under continuous grazing in ≈6 ha paddock without irrigation. Cows spent most of the day in the paddock (≈20 h) except during the milking.

Treatments and diet. Two treatments were evaluated: corn silage and CR silage. The feeding of the cows was based on grazing pastures (*Brachiaria brizantha*, *Brachiaria humidicola*, *Panicum maximum*) and typical shrubs of the region during the dry season (March 2019). Additionally, during the 20 days of the experiment, 1 kg of CR silage or corn silage (DM) were offered according to the treatment, as well as 300 gr of molasses and 100 gr of bran (cow d⁻¹) once a day during the milking in individual feeders. The amount of CR silage offered was according to the amount of corn silage offered by the farmer. Prior to the experiment, there was an adaptation period of 5 days, in which 0.25 kg of silage (DM) was provided daily, according to treatment. Silages intake was measure daily. Each week, approximately 1 kg of each of the silages was collected, as well as approximately 2 kg of pasture from the paddocks. The storage of the collected samples was carried out as described in experiment 1.

Chemical analysis of the silages. Samples from the CR silage, corn silage and grazed pastures were processed and analyzed as described in experiment 1. Hemicellulose was calculated by the difference between the NDF and the ADF. Table 3 shows the nutritional composition of the feedstuffs.

Table 3. Chemical composition of feed offered to cows in experiment 3.

Feed	Variable, % DM						
	DM	CP	NDF	ADF	Hemicellulose	EE	Ash
CR silage ¹	26.7	4.9	69.9	52.1	17.8	1.0	12.0
Corn silage ¹	24.2	5.0	66.6	42.6	23.9	1.5	7.2
Grazed pastures ¹	55.1	4.1	70.2	44.2	26.1	0.8	10.7
Molasses ²	77.9	8.5	0.4	0.2	0.2	0.2	13.3
Bran ²	89.1	15.0	26.1	13.1	13.0	15.2	10.4

¹ Data obtained from laboratory analyses according to AOAC [27] and Van Soest [28]. ² Data from NRC [26].
DM: dry matter, CP: crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber, EE: ether extract.

Milk production and composition. The cows were milked manually once per day, between 4:00 and 7:00 am. Milk production was measured individually over the last 10 days of the experiment. In the last 5 days of the experiment, 100 mL of milk from each cow was collected in plastic bottles and stored at -20°C until analysis. Milk samples were thawed with a water bath at 40°C , and then homogenized with a multipurpose shaker until reaching a temperature of 30°C . Milk composition analysis was performed in duplicate for each sample with a LactiChek TM-01 RapiRead® (Page & Pedersen International Ltd., Hopkinton, MA, USA).

Experimental design and statistical analysis. A completely randomized experimental design with 2 treatments and 10 repetitions was used. The results were analyzed with MIXED procedure of the SAS version 9.0 [30] including treatment, day and treatment * day.

3. Results and Discussion

3.1. Experiment 1. Physicochemical Evaluation of Silages with Different Levels of Cucurbita Argyrosperma Huber Residues

Figure 1 shows the results of the DM content, temperature, and pH of the CR silages. Significant differences ($p < 0.05$) were observed in the DM content (Figure 1A), as the results were close to the estimated DM content (Table 1). DM content of CR-77 and CR-59 was slightly higher (27.3 and 41.8%, respectively) than the estimated DM (25.41 and 40.17%, respectively); however, treatments CR-77, CR-71 and CR-65 had an adequate DM content for the silage process, which is between 20 and 40% DM. It has been reported that a DM content of less than 20% or greater than 40%, affects the silage conservation process [33]. This is consistent with López and Briceño [34] who have pointed out that silages with a DM content less than 20% are below the minimum parameters for a correct silage process. Furthermore, McEniry et al. [35] have noted that percentages above 40% of DM decrease the fermentation process. There were also significant differences ($p < 0.05$) in the temperature of the CR silages. In all treatments, the temperature was less than 30°C (Figure 1B). Wilkinson and Davies [36] mention that the deterioration of the silages begins when their temperature is above the environmental temperature, due to microbial oxidation of acids and water-soluble carbohydrates to CO_2 and water. In this experiment, environmental temperature was not registered, however, the average temperature during the time of year (September–October 2018) where the silages were stored was 28°C , with a maximum temperature of 32°C and a minimum of 24°C [37]. Borreani et al. [38] mention that high temperatures negatively affect the silage fermentation process, indicating that prolonged temperatures above 40°C cause protein denaturation, reducing the availability of amino acids. On the other hand, if the silage temperature is below 27°C , the growth of lactic acid bacteria, essential for initial fermentation, is slowed down [38]. Regarding pH, Figure 1C shows statistical differences ($p < 0.05$) between treatments CR-71 and CR-59. Several studies indicate that a pH below 5, represents adequate anaerobic stability and, therefore, it is a characteristic that demonstrates good quality in a silage [33,38–40]. pH is related to DM content and temperature; McEniry et al. [35] show

that a DM content above 40% increases the pH. These data are consistent with the results obtained with CR-59, which had a DM content of 41% and a pH of 4.4, which was higher compared to the other treatments. Regarding the relationship of pH with temperature, Kim and Adesogan [41] have noted that temperatures higher than 35 °C increase the production of compounds formed by pathogenic bacteria, which develop and proliferate at a pH greater than 5. It is important to highlight that, although a microbial count was not performed, the low pH could indicate a good silage process, inhibiting undesirable microorganisms.

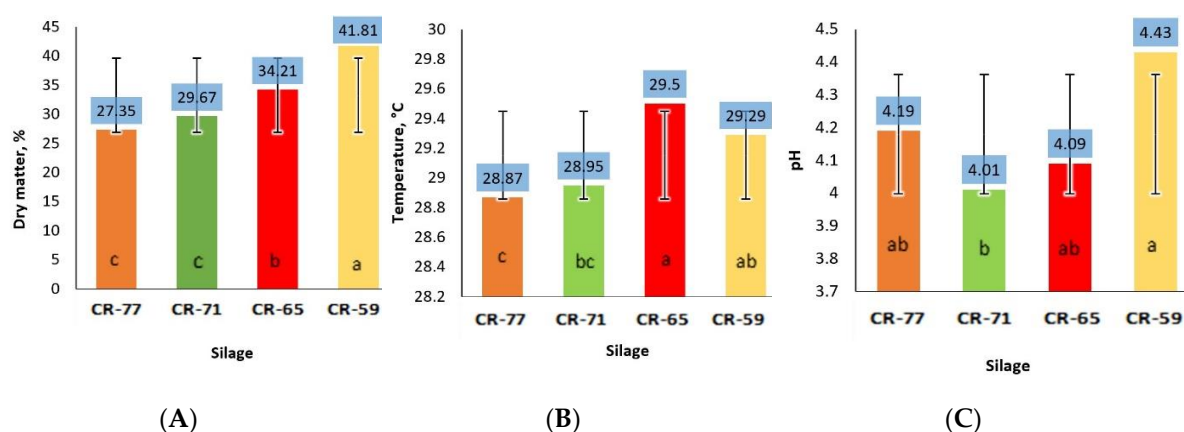


Figure 1. DM content (A), temperature (B) and pH (C) (mean $\pm$ SD) of silages made with CR in experiment 1. Within each figure, means with the different letter are significantly different ($p < 0.05$).

The results of the chemical composition of the silages are shown in Table 4. There were significant differences ($p < 0.05$) in the content of CP, NDF, ash and acetic acid. There were statistically significant differences ($p < 0.05$) in the CP content between CR-59 compared to CR-77 and CR-71. Thus, with the higher CR content, the silage had a higher amount of CP. In all the treatments, the CP content was lower than that reported in various silage studies with other varieties of pumpkin, such as with the Ishicu Kuru variety reporting 8% CP [22], or with the *Cucurbita maxima* species with 10.9% of CP [42]. Lorenzo-Hernández et al. [43] increased the content of CP up to 27% in CR and *Digitaria decumbens* hay silages adding urea. Dorantes et al. [13] reported that the dry residue of *Cucurbita argyrosperma* contains 8.6% CP, which is higher than the results reported in this study. The low CP content of the CR silages could be a consequence of the composition of the CR and the grass straw; the nutritional composition of a silage depends on the composition of its ingredients before ensiling [44].

Table 4. Chemical composition of CR silage in experiment 1.

Treatment	Item, % DM					
	CP	NDF	ADF	EE	Ash	Acetic Acid
CR-77	3.8 ^a	75.5 ^b	64.6	0.95	14.6 ^a	3.14 ^{ab}
CR-71	3.8 ^a	74.7 ^b	62.3	0.88	10.3 ^b	3.74 ^a
CR-65	3.4 ^{ab}	79.8 ^a	61.8	0.87	10.1 ^b	2.74 ^{ab}
CR-59	2.8 ^b	79.4 ^a	64.1	0.75	9.9 ^b	0.57 ^b
SEM	0.14	0.60	0.65	0.03	0.80	0.47

Within each column, means with different superscript letters are significantly different ($p < 0.05$). CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; EE: ether extract; SEM: standard error of mean.

Significant differences were found in the NDF content between CR-77 and CR-71 vs. CR-65 and CR-59 ($p < 0.05$), showing that NDF increases as the CR content decreases. The results obtained here differ from those reported by Lozicki et al. [45], who found that *Cucurbita maxima* and beetpulp silages, in an 80:20 ratio, showed around 30% of NDF, which represents a content well below the one reported

in this experiment (77.3% on average). The results reported by Lozicki et al. [45] can be attributed to the fact that the NDF content of the *Cucurbita maxima* fruit was 21.7%, considerably less than the 49.4% reported by Dorantes et al. [13] in the dry residue of *Cucurbita argyrosperma* Huber. However, the NDF content obtained in this experiment is similar to the NDF content in more conventional silages. For example, in a review by Chavira [46], it is indicated that sugar cane silages have around 78% NDF. According to the literature and the results obtained, the NDF content is closely related to the DM content of the ingredients used in the production of the silages. As mentioned in NRC, for beef cattle [31], the NDF content will be higher in by-products and plant residues (stems and straw), depending on the DM content. Regarding the ADF content, there was no significant difference ($p > 0.05$) between any of the treatments. In a previous study, Dorantes et al. [13] reported that the dry residue of *Cucurbita argyrosperma* Huber contains 40.4% of ADF, so in this study the high content of ADF may be a consequence of the straw from the grasses used to make the silages. Additionally, Lozicki et al. [45] reported that silages made with *Cucurbita maxima* and beetpulp contained 21.2% of ADF, so the ADF content of a silage depends on the nutritional composition of each pumpkin species. The ADF content in feedstuffs is indicative of digestibility, since the lower the ADF content, the greater the digestibility, and the higher the ADF content, the lower the intake [47]; therefore, silages made with CR are likely to have lower digestibility, which would negatively affect their quality.

The EE content showed no difference ($p > 0.05$) between treatments; however, the result obtained is considerably lower compared to than reported by Lozicki et al. [45]. In that study, a 3.3% and 3.7% EE content were reported in *Cucurbita maxima* and beetpulp silages, with and without inoculant, respectively. In other study, Dorota-Halik et al. [42] reported 4.6% of EE in silages made with *Cucurbita maxima*, which is also higher compared to the results in our study. On the other hand, significant differences were found in the ash content ($p < 0.05$) between CR-77 and CR-71, CR-65 and CR-59. This variation may be due to contamination with soil at the time of the collection of the CR [44]. It is well known that the percentage of ash in a food is related to its mineral composition [48]. Ash content has been reported in studies with various pumpkin species. Dorantes et al. [13] reported an ash content of 13.6% in the dry residue of *Cucurbita argyrosperma* Huber, and a Ca, P and Mg content of 1.1, 0.2 and 0.2%, respectively. Kim et al. [49], evaluated separately the husk, pulp and seed of various species of *Cucurbita* (*moschata*, *pepo* and *maxima*) and reported that the ash content was higher in *Cucurbita moschata* (10.3% in pulp and 13.9% rind) and the lowest in *Cucurbita pepo* (3.4% in pulp and 6.3% in rind). However, Hashemi and Razzaghzadeh [50] recorded an ash content of 14.3% in *Cucurbita pepo*. In studies carried out with *Cucurbita maxima* silages, an ash content of 6.8 to 7% has been reported [42], which is lower compared to the results obtained in our study. In silages with other agricultural residues (carrot, oats, corn and squash), the reported ash content is between 3.2 and 10.3% and among the main minerals found are Ca, P, Mn, Zn, Cu, Fe and Mg [22]; however, in the oat silage with broccoli the ash content found was from 27 to 29% [21].

Concerning acetic acid content, significant differences ($p < 0.05$) were observed between CR-71 and CR-59. CR-59 silage, which was elaborated with the lowest amount of CR and had the higher DM content, had an extremely and undesirable low content of acetic acid. According to Kung et al. [51], acetic acid content should be between 1 and 3% and is inversely correlated with DM content. With the exception of CR-71, our silages follow this correlation. On the other hand, López and Briceño [34] show that in legume silage, the acetic acid increases under optimal humidity conditions and carbohydrate availability. A higher content of acetic acid also stabilizes the anaerobic conditions due to its antifungal properties, thus preserving the silage for a longer time [51]. However the vinegar smell of acetic acid could affect consumption behavior, particularly when above 5% (DM) [52]. Propionic and butyric acids content were not detected in this experiment. The absence of these volatile fatty acids implies a good fermentation process and thus a good silage [51] as propionic and butyric acids are associated with the growth of undesirable and potentially harmful pathogenic bacteria such as *Clostridium* sp. [52,53]. In addition, values above 0.25% (DM) of butyric acid promote a rancid flavor [53].

3.2. Experiment 2. Evaluation of Preference Intake of Silages with Different Levels of *Cucurbita Argyrosperma* Huber Residues in Cattle

No significant differences ($p > 0.05$) were found in the intake of the different CR silages (Figure 2). However, it is important to note that the lack of statistical differences may be due to the low level of replication (four steers). There was also no effect due to the location of the treatments in the feeder or day ($p > 0.05$). The intake could have been influenced by the physical and chemical characteristics of the silages, or by the properties of the pumpkin residues. These results are similar to those reported by Razzaghzadeh et al. [54], who observed that the highest intake of a *Cucurbita pepo* silage by male buffaloes was related with the highest percentage of pumpkin residue (60%) within the silage. In studies with sheep, an intake between 645 and 652 g day⁻¹ of silages with 70% *Cucurbita maxima* has been reported [55]. A similar observation was recorded by Montes-Pérez et al. [56] with *Pecari tajacu* pigs, where the silage with a content of 76.4% of local pumpkin and 23.6% of ground corn, had a higher intake compared to treatments formulated with 50% of Taiwan grass (*Pennisetum purpureum*) and Ramón (*Brosimum alicastrum*), or a mixture of 50% pumpkin and corn. Intake was around 340 g day⁻¹ which led these authors to conclude that this preference is due to palatability, that is, the taste of the silage. In general, both the physical and chemical characteristics of a food affect its preference [57].

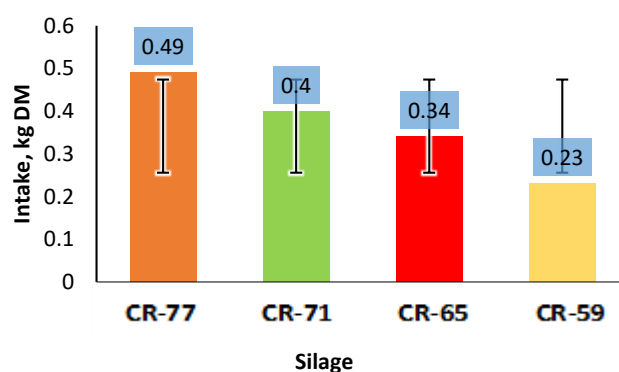


Figure 2. Preference intake of silages with different levels of CR in cattle (mean ± SD) ($p = 0.12$).

It is known that intake is affected by factors of the animal and the feed offered [58]. The exposure time to silages is related to voluntary intake and, in turn, to preference. In this case, the silages were offered to the steers for 20 min and the maximum intake was 500 g DM of material. No preference reports were found for the intake of pumpkin silage in cattle. Feeding preference in ruminants is affected by many variables that are likely to interact with each other, such as type of feeder, position of the feeder, odor, and taste of the food [32]. This agrees with Pinto-Ruiz et al. [59] who mention that the preference for some forage depends on the sense of smell and taste. Furthermore, with these senses, animals can detect toxic or anti-nutritional compounds, favoring the intake of other foods [60]. In our study, we found no differences ($p > 0.05$) in the position of the silages in the feeder, a result that contrasts with the report by Harper et al. [32], who mention that in the preference test they carried out for trying different flavors, the position affected the amount of food consumed, the time spent in feeding and the rate of intake in cows. Based upon the results from experiment 2 and with the aim to use most of the pumpkin residue which has no other use, we decided to use the composition of CR-77 treatment for the preparation of the CR silage for experiment 3.

3.3. Experiment 3. Evaluation of *Cucurbita Argyrosperma* Huber Silage in the Production and Composition of Cow's Milk in a Dual-Purpose System

Table 5 shows the results of silage intake, milk production and milk composition of cows in a dual-purpose system. There was a difference ($p < 0.05$) in the intake of corn silage and CR silage in the cows. The lower intake of CR silage by cows may be due to various factors, one of which is the adaptation to a new food, with a characteristic taste and odor that caused the feed not to be palatable.

We should note that this behavior could be due to the lack of molasses in the CR silage of experiment 3. Our silages from experiment 1 and 2 had molasses, however the farmer prepared his corn silage without molasses and thus CR silage for experiment 3 was prepared accordingly. Thus, it is likely that the absence of molasses in CR silage influenced the flavor of the silage, so it could be less palatable. Molasses contains non-structural (soluble) carbohydrates, mainly simple sugars, and disaccharides, which are rapidly fermented in the rumen. In the case of silages, the sugars present are fermented quickly and their content is low [26], so molasses is an additive commonly used in silage production as a source of nutrients for the bacteria responsible of the fermentation of protein silage [44]. On the other hand, it is likely that the 5 days adaptation period to the silages was insufficient for the cows to get used to a new food. Another aspect that affects consumption is rumen filling. It is known that if the NDF content of a forage is greater than 60%, it tends to decrease feed intake [47]. This is in line with the composition of the silages (Table 3), where we can observe that the NDF content is slightly higher in the CR silage (69.9%) than in the corn silage (66.6%). This is similar to the results described by Kendall et al. [61], who reported lower intake in cows fed with a TMR with 32% NDF compared to those fed a TMR with 28% NDF, suggesting that intake of feedstuffs was limited by rumen filling. However, taking into account that only 1 kg of DM silage was offered, it is unlikely that a lower consumption was due to rumen filling.

Table 5. Intake, milk production and milk composition of milk from cows fed with corn silage or CR silage (experiment 3).

	Treatment		SEM
	Corn Silage	CR Silage	
Intake, kg DM d ⁻¹	0.82 ^a	0.70 ^b	0.06
Milk yield, kg d ⁻¹	3.8	3.6	0.11
Milk composition, %			
Protein	3.27	3.27	0.01
Fat	2.45	2.07	0.10
Lactose	5.22	5.23	0.02
Solids non-fat	9.15	9.19	0.03

Within each row, means with different superscript letters are significantly different ($p < 0.05$). SEM: standard error of mean.

Although there was a lower intake of CR silage, there were no differences in milk yield between the two treatments (3.7 kg cow⁻¹ d⁻¹) (Table 5). Although milk production is low, these values are considered normal in dual-purpose production systems in the tropics. Sheen and Riesco [62] mention that milk yield in dual-purpose cows in the humid tropics, grazing with *Brachiaria* and a supplement of rice and beer residues was up to 5.2 kg d⁻¹, but decreased to 3.8 kg d⁻¹ when the supplement was not offered, indicating that the pasture does not cover the nutritional requirements of the cows during lactation. In another study with goats at grazing and at grazed pasture plus concentrate diet, it was observed that milk yield increased from 0.64 to 1.14 kg d⁻¹, respectively [63]. In Campeche, Mexico, 30% of cattle producers are engaged in dual-purpose systems. Nearly 80% of all cattle are crossbred between Zebu breeds and European breeds and the main form of feeding is based on grazed pasture, either continuous or rotational, and only 27% of producers use concentrated feed [64]. Due to the characteristics of dual-purpose systems in Mexico, it is understandable that milk production is low. It is important to highlight that the amount of silage offered during the adaptation and experimental periods, was one of the main limitations in experiment 3, which could have affected milk yield. In this experiment, the 1 kg DM cow⁻¹ of corn silage or CR silage was not enough to cover the nutritional requirements of the cows to increase their production. As observed in Table 3, silages and grass have a low CP content (<5%). According to the NRC [26], a CP content of 11.9% is required in the diet of cows with 454 kg of LW, in half lactation and 10 kg of milk per day. However, it should be noted that these requirements are for specialized European cattle.

There is no information regarding the use of CR silage in dairy cattle feeding and there are very few studies with other pumpkins. Halik et al. [65] reported increases of 2.5 and 6.3 kg in milk yield in cows fed 12 and 17% of *Cucurbita maxima* silage (replacing 40 and 50% of corn silage), in contrast with those fed without silage of *Cucurbita maxima*. In meat production systems, Razzaghzadeh et al. [54] mention that *Cucurbita pepo* silage does not affect feed intake and weight gain in buffaloes, concluding that up to 60% can be incorporated into the diet without causing any negative effect. Furthermore the inclusion of 70% silage of *Cucurbita maxima* in the diet increased weight gain in lambs [46].

Regarding the composition of the milk, there were no differences ($p > 0.05$) between treatments (Table 5), this is probably a consequence of the fact that only 1 kg DM of silage was offered. Halik et al. [65] reported a lower fat content but a higher protein and lactose content in the milk of cows fed 12 and 17% *Cucurbita maxima* silage; however, they did not report the intake of silage or the total diet. In our study, the low-fat content is remarkable. According to Mexican standards NMX-F737-COFOCALEC-2016 and NOM-155-SCFI-2012 [66,67], the minimum fat content in milk must be 3%, consequently the composition of the milk in our study does not comply with the specifications of national regulations. However, the dual-purpose systems relying on grazing are dependent on the quality and quantity of available forage, thus affecting milk quality and characteristics [68]. The low-fat content in milk is probably the consequence of a low feed intake and its low nutritional value. Bedoya-Mejia et al. [69] mention that the lipid component is recognized as the most important in milk in terms of cost, nutrition, and the physical and sensory characteristics of the product. In general, animals fed nutritionally adequate diets produce more milk, with a higher level of fat and protein than animals with less nutritional quality feed [70]. Therefore, it seems likely that silages and pasture did not covered the nutritional requirements of cows to produce milk with higher nutritional quality.

In Campeche, Mexico during 2019, 19,047 ha of *Cucurbita argyrosperma* Huber were harvested [14] with an estimated yield of 1.1 t de MS/ha [13] and thus an estimated production of 20,856 tons of DM of CR, which were left in the fields after seed harvesting. Considering the increasing demand of food from animal origin [5], it is primordial to search for feed alternatives for livestock in order to diminish feed–food competition [8,9]. Our previous studies [13,16,17] and others [43] have described the potential of CR for animal feeding. We demonstrate that CR can be ensiled, and despite the technical limitations, this is the first pilot study in vivo suggesting that CR is accepted by cattle and does not modify milk production or composition in cows in a dual-purpose system.

4. Conclusions

Despite the technical limitations, the current pilot study shows that it is feasible to properly ensile the CR, and consequently represents an alternative feedstuff for cattle in Campeche, Mexico. It is possible to use CR silage as feed for livestock during the dry season, when the availability of forage decreases, and at the same time, take advantage of an agricultural residue not suitable for human consumption that has no other use, reducing feed–food competition. Additionally, the use of crop residues favors the economy of small-scale farmers by reducing the amount of concentrates in livestock feed. Environmentally, the silage of CR diminishes the amount of organic waste. Therefore, it would be plausible to use millions of tons of CR produced annually as feedstuff in Campeche, Mexico. However, it would be convenient to increase the CP content of CR silages to satisfy the nutritional requirements of cattle in tropical regions. In addition, it is necessary to carry out more studies increasing the amount of silage offered to animals and to include molasses in the elaboration of CR silage to improve palatability.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2071-1050/12/18/7757/s1>. Supplementary material S1: Elaboration of silages with *Cucurbita argyrosperma* Huber residue in experiment 1; Supplementary material S2: Design of the feeders in the preference test of experiment 2; Supplementary material S3: Elaboration of the *Cucurbita argyrosperma* Huber silage in experiment 3.

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