Aerobic semi-solid fermentation of *Opuntia megacantha* as feed supplement and its effect on dairy cows (Holstein)

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ABSTRACT

Fermented prickly pear (*Opuntia megacantha*) has been used as forage in goats and sheep. Thus, the objectives of this study were to assess bimonthly production of cladodes var. Narro, compare the quality of those fermented versus non-fermented, and assess the effect of fermented cladodes in milk production of Holstein cows in stables (La Partida, Matamoros, Coahuila, México). Central pivot irrigation was used to water prickly pear plantation with a density of 17,500 plants ha^{-1}. To assess cladode quality (proximal analysis) before and after fermentation, a completely randomized bifactorial design was used -non-fermented (N-0) and fermented (N-F) cladodes- with three replicates each. To assess the fermented cladode effect in milk production, a completely randomized design was established with first-calving Holstein cows where each one represented one replicate. The treatments were two diets, Dnor (normal) and Dnop (fermented cladodes). The results showed sufficient bimonthly production (14,380.8±1,676 kg t ha^{-1}) of fresh prickly pear leaves for daily bio-digestor functioning (200 kg). Crude protein content and metabolizable energy showed significantly greater and contrasting values compared with non-fiber carbohydrate content (sugars and starch), showing a significant decrease in fermented cladodes with respect to non-fermented. Other proximal components did not show significant differences between both N-F vs N-0 cladodes. Average milk production increased when cows changed from Dnor (26.08 ± 4.8 L Day^{-1}) to Dnop (30.07 ± 5.3 L Day^{-1}) diets, recording three groups with less, medium, and greater significant increase in milk production. No correlation was found between production and increase.

Keywords: Biotechnology; forage; protein; animal production; milk cows.

INTRODUCTION

The Comarca Lagunera (comprising part of the states of Coahuila and Durango, México) is the main dairy belt of Mexico with the greatest milk production at national level. In 2018, billions of liters (2,448,598) were produced equivalent to 20.4 % of the total production in México, which was 12,008,239 billion L (SIAP, 2020). A greater impact of global warming effects exists in this region, such as drought and increase in heat, causing losses for cattle raising with a major effect on especially vulnerable livestock as dairy cows (Curiel-Ballesteros, 2012). This problem has become more pronounced due to the association between limited forage availability and high cost of protein sources, which impact on deficient animal nutrition (Murillo-Amador *et al*., 2009). The prickly pear (*Opuntia spp*) is a vegetable resource with high development potential in the arid regions of Mexico, especially in the Comarca Lagunera because its anatomical and physiologic characteristics -attributed to crassuleacea acid metabolism (CAM)- grant it a wide adaptation rate (Andrade *et al*., 2007) and allow it to progress as a high production crop with low hydric requirements (Flores-Hernández *et al*., 2005; 2007). The prickly pear cladode or leaf -as a perennial wild resource- is available the whole year round and used as auxiliary forage in drought season because of its high-water content (70 to 90 %). According to López-González *et al.* (2003), its protein content is low (2.7 to 8.8 % in dry matter), which limits its use as forage. Nonetheless, in countries -as Brazil (semi-arid northeastern) with a larger surface at world level of prickly pear forage in traditional and intensive cultivation (600,000 ha)- the spineless prickly pear leaf, cut and chopped is used for direct livestock consumption (Torres-Sales, 2010).

Solid and semi-solid fermentation has been performed for many years in vegetables and/or their waste to increase protein content in feed and/or improve their physical characteristics, such as color, aroma and/or flavor. For example, Koji is obtained by culturing the fungus *Aspergillus oryzae* on cooked cereals Shoyu, Miso, and Ontjom (Hesseltine, 1972). This fermentation process has been used efficiently to produce feed for animal consumption once their physical and chemical properties have been determined (Preston, 1985). Semi-solid fermentation in prickly pear (*Opuntia* spp) is performed on a substrate of chopped prickly pear leaf adding a mineral mixture to increase protein content (Tabosa, 2003). In intense forage, protein production of microbial origin -formed by starting from fermentation or microorganism growth- is useful on vegetable substrates rich in carbohydrates and fiber (Ariosvaldo *et al.*, 2004). Fermentation characteristics differ much in the use of microorganisms, which may be natural from vegetable substrate or selected microorganisms, such as fungi, bacteria, or both, as well as nutrients or chemical compounds used as nitrogen source to favor growth of these microorganisms and with microbial protein increase (Elías and Lezcano, 1993; Gomes da Silva *et al.,* 2007; Miranda-Osorio *et al*., 2009).

A technology was developed in Mexico, whose process of prickly pear protein enrichment was based on semi-solid aerobic fermentation. This equipment showed advantages because all the process was mechanically performed, consequently, saved time and labor; additionally, a unicellular fungus (*Sacharomyces cereviceae*) and two nitrogenous sources, urea and ammonium sulfate were used as nutrients (Flores-Hernández *et al*., 2017a). The fermented prickly pear of different cultivars and varieties -applying the described technology- has been used to feed goats and assess consumption and weight gain (Flores-Hernández *et al*., 2017b), as well as in sheep to assess daily, weekly, and monthly weight gain (Flores-Hernández *et al*., 2019). Therefore, the objectives of this study were to assess bimonthly production of cladodes of the var. Narro (spineless), compare the quality of those fermented vs non-fermented, and assess the effect of fermented cladodes in milk production of Holstein cows in stables.

MATERIALS AND METHODS

Study area

The research was performed in the State of Coahuila, México in the experimental field and commercial stable La Partida, Matamoros, located at 25° 34´ 57" N and 103° 17´ 54" W, at an altitude of 1120 m.a.s.l. in 2018. According to the National Meteorological Service in Mexico, the normal historical climatological in this site is characterized by being dry much of the time, with a minimum and maximum annual temperature from 15.2° to 30.1°C, respectively, a total annual precipitation and evaporation of 382.4 mm and 1900.1 mm, respectively.

Cladode production

For the fermentation and feeding process for cattle, the cladodes used were of the Narro variety (*Opuntia megacantha*). This variety is a spineless prickly pear with high biomass production and light green color. The cladodes were obtained from a plantation established in June 2017. They were watered by central pivot irrigation system with a density of 17,500 plants ha^{-1} sown in beds or plots of four rows or furrows in a staggered plantation design at a distance of 0.50 cm between plant and rows and a space of 2.50 m in width between beds or plots. In December 2017, formative pruning at ground "first level" was performed, that is, the mother leaf (leaf initially planted) and two leaves in production, commonly called "rabbit ear". In April 2018, cuttings started from the cladodes in production with a total of four cuttings.

Cladode fermentation process

The harvested cladodes in field were transferred to a warehouse located in the study site facilities, whose average temperature during cladode storage was 27 ± 0.2 °C. The cladode fermentation process was performed in a bio-digestor machine (Title No. 2641- IMPI, MX) according to the process (Title No. No.339696- IMPI, MX), which consisted of cutting cladodes in fractions that dropped to the mixer cylinder (homogenizer) by gravity. It was then used as substrate, first adding urea (1.0 %) and ammonium sulfate (0.10 %), used as nitrogen source for the development of fungus population or dry yeast (*Sacharomyces cereviceae*) and added to the mixture in 1 %. *Saccharomyces cerevisiae* (also known as "Baker's Yeast" or "Brewer's Yeast") is a unicellular fungus responsible for alcohol production and bread formation.

Fermentation started with eight interspersed cycles (1/2 h of aeration movement and 1/2 h of rest) for eight hours (programmed by timer). The process started at 08:00 h and ended at 16:00 h. In the first day of each week, 1 kg of dry yeast was used to start fermentation, and in the subsequent days, 3.78 L of prickly pear juice from the previous fermentation were used. This juice was obtained immediately after fermentation and stored in refrigeration (6 to 8 °C) for its use the next day. Weekly yeast recycling was performed to decrease process costs and increase feasibility. Temperature and substrate pH (Conductronic PH10, CDMX, MX) were measured at the beginning and end of fermentation.

Proximal analysis: non-fermented and fermented cladodes

For the fermentation process, chopped cladode samples were taken before (N-0) and after (N-F) the fermentation process at days first (NF-1), third (NF-3) and seventh (NF-7) for three consecutive weeks. Subsequently, a proximal analysis was performed to evaluate crude protein (CP), which was analyzed according to the CP process of animal feed (990.03, AOAC, 2005b). The metabolizable energy (ME Mcal kg^{-1}) and total digestible nutrient percentage of dry matter (% DM) were measured according to McDonald *et al*. (2011). The non-fibrous carbohydrates (NFC, sugar, and starch) and neutral detergent fiber (NDF) (% DM) were determined by the method of Van Soest *et al*. (1991). The minerals were analyzed according to the ash process (C) of animal feed (942.05, AOAC, 2005a). *Opuntia* DM was measured by the partial DM process adapted from Goering and Van Soest (1970). The acid detergent fiber (ADF) (% DM) was measured according to the process for ADF and lignin in animal feed (973.18, AOAC, 1997). All analyses were performed at Agrolab (certified laboratory of Dr. Martin J. Traxler) in Gómez Palacio, Durango, MX.

Effect of fermented cladodes on milk production

To assess the effect of prickly pear fermented cladodes on milk production, 12 first-calving Holstein cows were used with average age and measurements of 39.52 ± 3.42 months, height of 150.83 ± 3.51 cm, weight at the start of the experiment of 577.92 ± 81.54 kg, and feed consumption of 24 kg based on DM. To feed the cows, two diets were used -normal diet (DNor) based on the components used in the referred stable (Table 1). This diet was supplied for 30 days, and daily milk production was quantified. Milk production was not recorded for the first 72 h previous to starting diet number two (Dnop), which consisted of supplying the fermented cladodes. This diet was made by modifying the normal diet and also supplied for 30 days quantifying daily milk production. In both diets, calculus was performed to make proximal protein content and dry weight equal. In the DNop diet, a small bale portion of alfalfa and pre-mixture were eliminated. Chopped alfalfa and corn (second silage) were completely eliminated from this diet, and only was corn (first silage) increased in low proportion with respect to the Dnor diet. When the cost per diet calculus was performed, the prickly pear diet (Dnop) showed a low cost with a difference in favor of 2.83 USD, with respect to the normal diet (Dnor). Both diets were provided *ad libitum* (dry weight 24 kg according to 4% average live weight).

Experimental design

To assess quality (proximal analysis) of the cladodes before and after the fermentation process, a completely randomized bifactorial design was used. The two treatments were non-fermented (N-0) and fermented (N-F) cladodes with three replicates each. The average values of all independent variables (*Opuntia* dry matter, crude protein, metabolizable energy, total digestible nutrients, non-fibrous carbohydrates, neutral detergent fiber, minerals (ash) and acid detergent fiber) were considered statistically different when $p \leq 0.05$.

To assess the effect of fermented cladodes on milk production a completely randomized design was established with repeated measurements in time, with 12 first-calving Holstein cows where each cow represented one replicate. The treatments consisted of two diets, DNor (normal diet) and DNop (fermented cladode diet) with the components described in Table 1.

Table 1. Diet components used in the normal diet (DNor) in fresh weight and fermented *Opuntia* (DNop) diet in feed of Holstein cows. La Partida, Coahuila, Mexico.

Diet components	Normal diet (DNor)			Fermented *Opuntia* (DNop)		
	DM (%)	kg per cow	%	DM (%)	kg per cow	%
Rye grass	20	12	26.049	20	12	26.63
Bales of dehydrated alfalfa	90	1.11	2.45	90	0.89	1.97
Chopped dehydrated alfalfa	60	0.67	1.47	---	---	---
Forage oats in bale	90	0.45	0.99	90	0.45	1.0
Rolled fodder corn	86	6.41	14.61	86	6.41	14.23
Corn silage 1st	36	11.78	26	36	14.72	32.67
Corn silage 2nd	36	2.94	6.5	---	---	---
Premix	90.65	9.94	21.94	90.65	9.72	21.57
Fermented *Opuntia*	--	--	--	91.5	0.87	1.94
*Total normal diet	---	45.30	100.009	---	---	---
**Total fermented *Opuntia*	---	---	---	---	45.06	100.01

** = actual weight of diet 45.30%, dry-weight 24.03%. Price per cow $121.58.*
***= actual weight of diet 45.06%, dry-weight 24.03%. Price per cow $118.75. Roller fodder corn = Rolled corn is the result of subjecting the grain to a process of gelatinization of the starch cells, which allows increasing digestibility. Premix = "concentrated" food for milk (mix of different ingredients). Corn silage 1st = this silage contains cobs; Corn silage 2nd = this silage does not contain cobs.*

Statistical analysis

Bartlett's test was performed on the data to test homogeneity of variance. Data were compared using univariate analysis of variance (ANOVA) according to a one-way experimental design. The differences among the means of variables associated to the proximal component analysis of the diets were determined by Tukey's honestly significant difference (HSD) multiple range test at $p \leq 0.05$. A Pearson's correlation ($p \leq 0.05$) analysis was performed among all the variables of the proximal component analysis.

The differences within variables between diets were made by orthogonal contrasts ($p \leq 0.05$), that is, independent comparisons of means. The first contrast was made by comparing diet 1 (Normal diet -control diet-) versus diet 2 (Fresh fermented *Opuntia* -experimental diet). In all cases, mean values were considered significantly different when $p \leq 0.05$. The statistical analyses were performed using Statistica v. 13.5 (TIBCO Software Inc., 2018). The difference in milk production (percentage of variation) per cow was calculated using the formula:

$$Percentage\ of\ variation = \frac{\text{Final milk production}}{\text{Initial milk production} - 1}\ x\ 100 \quad (1)$$

This formula demonstrated an increase or decrease, depending on the case, of the production between both months. In addition, the Pearson's correlation index was determined, assuming that the four variables recorded per cow were linearly related to the increase in daily and monthly milk production.

RESULTS AND DISCUSSION

Cladode production

Cladode production of the var. Narro was considered as bimonthly production (shoots in February and evaluation in April), harvesting only the excess of leaves conserving the formative pruning of the "first floor" (Fig. 1). The var. Narro is a cultivar composed of four varieties of similar characteristics in the northern regions of Mexico and shows a greater variation because of its differences in origin, which is desirable since it grants greater ecological plasticity without affecting its production. Total production per plant was 0.848 ± 0.113 kg and 0.0635 ± 0.0085 kg in fresh and dry weight, respectively, which extrapolated to a hectare, a production of 14,380.8 ± 1,676 kg and 1,077.12 ± 125.56 kg was estimated in fresh and dry weight, respectively. The production in dry weight was sufficient to provide cladodes per month to the bio-digestor used since the operation capacity of the bio-digestor was 200 kg daily of fresh prickly pear with a monthly demand of 6.0 t. Cladode production may increase if growth is allowed to a "second floor" (Fig. 1) in the mother plant. Moreover, the plantation management has positive effects in cladode production when it is fertilized with organic matter (1 kg planta^{-1} of manure) at least once a year after the formative pruning and before regrowth. Estimated dry matter bimonthly production was 1.07 t/ha^{-1} with an estimated annual production of 6.4 t/ha^{-1} which was less than that reported by Torres-Sales (2010) in forage prickly pear in the traditional system, whose productions were 30 to 50 t/year^{-1} and intensive 60 t/year^{-1} with high density population.

Figure 1. *Opuntia* plant design to cladode production with a "first" or "second floor".

Proximal analysis: non-fermented and fermented cladodes

Crude protein, metabolizable energy, and non-fibrous carbohydrates showed significant differences ($p \leq 0.01$) between non-fermented versus fermented cladodes (semi-solid aerobic fermentation). The crude protein content was greater in fermented cladodes with an average value of 36.55 %, which was superior to the values reported for non-fermented cladodes that ranged from 2.7 to 8.8% according to López-González *et al.* (2003). The high protein content in non-fermented prickly pear was attributed to two reasons. The first one was the distinctive characteristic of the variety used, and the second one because the prickly pear cladodes used were two-month age old, same which in general showed a greater protein content since they were in full growth with respect to the annual cladode formed or oldest cladode in age known as suberized cladode or leaf (Flores *et al*., 1995).

In contrast, non-fibrous carbohydrate content (sugars and starch) showed a significant decrease in fermented cladodes. A significant but negative correlation (-0.96, $p \leq 0.05$, n=12) was observed between crude protein content and non-fibrous carbohydrates. The response to crude protein increase correlated to carbohydrate decrease has been reported as a normal condition of the fermentation process due to population growth of the yeast (unicellular fungus) that requires carbohydrate consumption of the medium or substrate (Hernández-Peñaranda, 2003). In oxygen presence, the yeast grows efficiently starting from carbohydrates and nitrogen of the medium to produce biomass (protein) and CO_2 (Hernández-Peñaranda, 2003). The metabolizable energy also increased in the fermented cladodes, which had a nutritional benefit. It showed a value of 2.56 Mcal kg^{-1} that went beyond pasture energy showing an average value of 2.08 Mcal kg^{-1} and similar to alfalfa that showed 2.64 Mcal kg^{-1} (Felker, 2003). According to Van Soest (1994), the fermented cladodes adjust to a CP feed of > 20%, ACD < 31%, and NDF < 40% that results in 151 points, catalogued as supreme foraging quality. Recently, Flores-Hernández *et al*. (2019) using the Villanueva variety reported that fermented cladodes significantly ($p \leq 0.01$) decreased carbohydrate content, increased crude protein content and energy compared with unfermented cladodes. Flores-Hernández *et al*. (2017) reported that the enriched cladodes showed greater crude protein with respect to alfalfa (hay), sorghum, and non-fermented cladodes (natural prickly pear) through semi-solid fermentation with the commercial dry yeast (florida brand) *Saccharomyces cerevisiae* at 1% and ammonium sulfate at 2%.

The enriched cladodes showed non-fibrous carbohydrates with respect to sorghum and alfalfa hay, which favored the fermentation process both external (protein enrichment) as internal (ruminal fermentation), increasing its foraging and nutritional quality. In the fermentation process used, yeast was weekly recycled during the 3-week length of the study. This recycling consisted of reutilizing the previously fermented juice, which allowed a considerable saving in the process since the cost of dry yeast was approximately 5.00 USD for each 100 kg of cladodes. Previous studies (Flores-Hernández *et al*., 2017 a,b, 2019) have demonstrated that using "new" yeast weekly did not have a significant effect in the fermentation process. According to García-Cortés (1995), the environmental conditions of fermentation should be considered since they may affect recycling because the optimum value of *Saccharomyces cerevisiae* development is of 4.5 ± 0.5 to a temperature of 25.5 ± 3.5 °C. In this study, temperature (27 ± 0.2 °C) and pH (6.2 ± 0.5) were recorded during the week, so the conditions were considered slightly higher than the normal limits for fermentation, without significant effect on the amount of the resulting protein.

Table 2. Proximal analysis of fresh *Opuntia* sp. var. Narro cladodes before (N-0) and after semisolid fermentation process (N-F) for three weeks. The fermented cladodes were used to feed Holstein cows for one month. La Partida, Coahuila, Mexico.

Components	Before (N-0)	After (N-F)
Crude protein (% DM)	10.42 ± 1.79 b*	36.55 ± 4.82 a
Metabolizable energy-ME (Mcal kg^{-1})	2.15 ± 0.07 b	2.56 ± 0.092 a
Non-fibrous carbohydrates-NFC (% DM)	32.39 ± 1.89 a	10.22 ± 4.29 b
Acid detergent fiber-ADF (% DM)	19.76 ± 1.58 a	19.71 ± 2.69 a
Neutral detergent fiber-NDF (% DM)	23.46 ± 2.42 a	21.37 ± 1.66 a
Total digestible nutrients-TDN (% DM)	58.91 ± 2.10 a	61.38 ± 2.17 a
Minerals-Ashes (% DM)	28.93 ± 1.45 a	27.73 ± 1.45 a
Cladodes dry matter (%)	7.99 ± 0.33 a	7.95 ± 0.28 a

**Values represent the mean ± standard error. Values in the same row followed by different letter differ statistically (Tukey's honestly significant difference (HSD), p ≤ 0.05).*

Effect of fermented cladodes in milk production

Milk production showed significant ($p \leq 0.001$) differences between diets with greater production when cows were fed with the fermented cladode diet (Table 3). The increase in milk production was attributed to the fresh and high live yeast (*Sacharomyces cereviceae*) supply - product of its multiplication by aerobic fermentation converted to a greater protein content as a result of rumen fermentation due to a decrease in oxygen- while using the diet based on fermented prickly pear. This situation favored anaerobiosis and stimulated cellulolytic bacteria, improving the animal productive response, which agreed with that reported by Suárez-Machín *et al*. (2016). In this same sense, Rivas *et al*. (2008) mentioned that the use of *Sacharomyces cereviceae* was recommended for milking cows during the first 105 days postpartum because its improved milk and fat production by the effect of stimulating action of the yeast in the rumen and a greater availability of nutrients for the mammalian gland.

The daily average production of the animals in study was 28.11 ± 5.52 L day^{-1} with a maximum value of 49.20 and minimum of 8.40 L day^{-1}. These values were considered high if compared with the average daily production in the region of study, which was 14.3 L d^{-1} (SIAP, 2020). The change in diet caused and increase in milk production among the cows used ($p \leq 0.000001$), which allowed classifying milk production in three categories, low, medium, and high (Table 3). According to daily increase in milk production in cows fed with fermented prickly pear and according to the medium rural price per liter of milk in the region -(during 2019) 0.28 USD/L^{-1} (SIAP, 2020)- a daily average profit per milk liter was expected of 0.34 USD, 1.18 USD, and 1.93 USD in milk production levels: low, medium, and high, respectively, adding savings of 0.13 USD per cow achieved by changing from normal to fermented prickly pear diet. Nonetheless, the three-category classification and categorical cow selection with greater milk production derived from fermented prickly pear feed were not adequate since no significant correlation was found between milk production in cows fed with the normal diet and increase in milk production when fed with the diet based on prickly pear.

The results showed that the cow with ear tag No. 3700 fed with the normal diet and an average daily production of 36.3 ± 3.7 L day^{-1} showed an average daily increase of 4.3 ± 1.7 L when fed with fermented prickly pear. Whereas the cow with ear tag No. 6139 fed with the normal diet and an average daily production of 24.01 ± 2.98 L showed an average daily increase of 5.28 ± 3.66 L day^{-1} when fed with fermented prickly pear. These results produced numbers of 10.16 USD and 6.72 USD daily average, respectively, in the two types of production, both with the same feed cost (6.08 USD day^{-1}) with respect to the normal diet (DNor) according to the average milk price per liter paid to the producer (0.28 USD). Thus, the profit varied from 4.08 USD to 0.64 USD, respectively, which highlights the importance of an adequate balance of expense per diet to achieve a greater milk yield with a lower cost. This balance should estimate the economic advantages of the increase of fermented prickly pear since this diet used 4 kg of humid-based prickly pear that corresponded approximately to 400 g dry-based (2% of daily feed), which may increase to 20 or 30% in the diet. With this respect, prickly pear (non-fermented) may be used at 30% (dry matter) in feed for milk Holstein cows with medium to high production. However, taking milk production, changes in corporal condition, and food consumption into account, 20% could be the most adequate level according to González *et al*. (1998). The use of fermented prickly pear mixed with other ingredients may be a common practice in milking stables since in this manner diet composition is regulated and allows having a total ration mixed, which is recommended by Van Soest (1994). In this sense, Pessoa *et al*. (2004) reported that the diets that consist of prickly pear, sorghum silage, and concentrate should be provided in a complete mix. According to De Andrade-Ferreira *et al.* (2012), prickly pear is shown as vital forage cultivation for sustainability of agricultural systems in semi-arid regions, mainly as a source of energy, which is why it should be adopted effectively.

Although the results of this study are relevant in milk production cows fed with fermented prickly pear because it may lower costs or in its case increase the ratio of fermented prickly pear in the diet as milk production increases, further studies should widen the period of feed and milk production evaluation. It is important to consider that cows usually require a period of approximately two to three weeks to adapt to the feed, which also has a bearing on milk production stage according to González *et al*. (1998) and Sepúlveda-Varas *et al*., 2017).

Table 3. Milk production and increase in Holstein cows fed with normal and fermented cladode diets. La Partida, Coahuila, Mexico.

Groups	Milk production (L day^{-1})	Increase (L day^{-1})
Low	27.16±5.58 b*	1.16±0.84 c*
Medium	31.77±5.00 a	3.99±0.45 b
High	31.27±5.45 a	6.56±0.88 a
	Diets	**Milk production (L day^{-1})**
	Normal diet	26.08±4.8 b*
	Fermented *Opuntia* diet	30.07±5.3 a

**Average values with the same letters in the same column do not differ statistically (independent or orthogonal contrast comparisons, p ≤ 0.05). Values represent the average of 12 replicates ± deviation standard.*

CONCLUSIONS

The bimonthly production of 14.6 t ha^{-1} of fresh prickly pear leaf *Opuntia* var. Narro (spineless) with 17,500 plants/ha^{-1} is considered sufficient for daily function of the bio-digestor (200 kg).
The content of crude protein and metabolizable energy showed significantly greater values. Contrastingly, the non-fibrous carbohydrate content (sugars and starch) showed a significant decrease in fermented cladodes with respect to those non-fermented.
The average daily milk production increased from 26.08 ± 4.8 L to 30.07 ± 5.3 L when cows were fed with the diet of fermented cladodes. Three groups were recorded with lower, medium, and high significant increase in production. No correlation was found between production and increase.

ETHICS STATEMENT

In this study, milk samples were taken from Holstein cows from a private stable. The animals were stabled with conventional management applied to stables in the area of study. The animals did not receive any mistreatment or treatment that may put their life in risk or stress due to inadequate management.

CONSENT FOR PUBLICATION

This research study does not contain any individual person's data in any form (including any individual details, images, or videos).

AVAILABILITY OF SUPPORTING DATA

All data generated or analyzed during this study are included in this published article.

COMPETING INTERESTS

The research has no financial or commercial purpose that must be interpreted as a potential conflict of interest in the future.

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AUTHOR CONTRIBUTIONS

Conceptualization, AFH, FJMR, CMH and GGH; methodology, OEA, GGH and CHB; formal analysis, BMA; investigation, OEA and CHB; resources, AFH; data curation, writing-original draft preparation, AFH, FJMR and CMH; writing-review and editing, BMA; project administration, AFH; funding acquisition, AFH and BMA.

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