

## Preconditioning For Export In Charolais Calves With Diets Added With Nopal (*Opuntia Ficus-Indica*): Productive And Hematological Response

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### ABSTRACT

The objective of the study is to evaluate the addition of two sources of nopal (*Opuntia ficus-indica*) in diets for preconditioning of Charolais calves for export with regard to productive and hematological parameters. A total of 30 weaned calves of 180 days of age, with a live weight (BW) of 155.53 kg, were assigned to three homogeneous treatments. T1) Control (n = 10; 154.05 ± 3.26 kg BW; fed a base diet (DB) consisting of corn stubble, rolled corn, dry distillers' grain, urea, probiotics, and molasses; T2) DBNN (n = 10; 155.79 ± 2.26 kg BW), fed with DB + 10% natural prickly pear; T3) DBNE (n = 10; 156.75 ± 3.10 kg BW), fed with DB + 10% protein-enriched prickly pear. The alterations in PV and daily weight gain (GDP) were meticulously observed, in conjunction with a comprehensive array of hematological parameters. These included leukocytes (uL), segmented neutrophils (%), lymphocytes (%), mixed cells (%), segmented neutrophils (uL), lymphocytes (uL), and mixed cells (uL), erythrocytes (uL), hemoglobin (g/dL), hematocrit (%), mean corpuscular volume (MCV, fl), mean corpuscular hemoglobin (HCM, Pg), mean hemoglobin concentration (MHC, g/dL), platelets (uL), and cortisol (ug/dL). Subsequent analyses revealed no statistically significant differences between the treatments for PV and/or GDP (p > 0.05). The hematological variables, HCM and MCV, exhibited significant differences (p < 0.05 and p < 0.10, respectively), with the highest values recorded in the control group at the conclusion of the test. The cost-benefit analysis revealed that the DBNN and DBNE were 26% and 29% more economical than the conventional base diet. Therefore, it is concluded that the inclusion of 10% natural and/or enriched nopal in the diets of preconditioning export calves is an economically viable option. This practice does not affect the productive response or the health of the animals.

**Keywords:** Meat, nutrition, cattle, growth

### INTRODUCTION

In the 2022–2023 cycle, Mexico exported 901,754 head of live cattle to the United States, marking a 10% increase from the previous cycle. Of these exports, 79% were male calves. In this cycle, the majority of the cattle (430,778 heads) were exported through the customs office of San Jerónimo, Chihuahua (SADER, 2023). This information provides a context for the increase in demand for live cattle from developing countries, which will continue to grow due to the increase in the world's population (Sijpestijn et al., 2022).

In Mexico, calves undergo a preconditioning period prior to exportation to the United States. During this period, which lasts 30 to 45 days, the animals are kept in handling pens before being crossed into the United States for sale (Beef Cattle Research Council, 2025; Dhuyvetter et al., 2005). This practice has been demonstrated to mitigate the stress associated with moving the pens of origin and to enhance the welfare and mood of the animals (Kvamme et al., 2024). However, it has been reported that shorter periods (21 days) may be sufficient to maintain calf productive performance, as well as meat quality, while reducing feed and handling costs, compared to traditional programs of up to 42 days (Anderson et al., 2016). However, the export protocol currently in effect for Mexico stipulates that calves must have remained free of sanitary restrictions for a minimum of 60 days within the national territory prior to exportation. This protocol entails prolonged periods of handling, feeding, and sanitary surveillance, which can result in increased operating costs (USDA-APHIS, 2025).

According to the regulations, feed constitutes one of the most significant expenses in beef cattle production, underscoring the paramount importance of nutrition as a pivotal consideration for producers (Moore et al., 2008). It has been demonstrated that the judicious use of feedstuffs can lead to a reduction in production costs, thereby generating a greater economic return (Arthur & Herd, 2008). In a similar vein, the immune system of cattle is influenced by their nutritional status (Carroll & Forsberg, 2007). Consequently, diet constitutes the primary modifiable factor influencing health outcomes, as it supplies the nutrients essential for an animal's maintenance, growth, and immune system function (Palomares, 2022).

In arid and semi-arid regions of northern Mexico, livestock feed is contingent on the availability of fodder, a resource that is both scarce and expensive. In this context, the prickly pear cactus (*Opuntia*) emerges as a viable alternative due to its low cost and low water requirements for its production (Salem & Smith, 2008; Salem & Abidi, 2009; Meza-Herrera et al., 2017). However, information regarding the utilization of nopal in dietary interventions for the preconditioning of calves for export in Mexico remains severely constrained.

The objective of this study was to evaluate the addition of two sources of forage nopal to diets for preconditioning Charolais calves for export and its effect on productive and hematological parameters. This objective was pursued in order to develop feeding alternatives that reduce costs without affecting the productivity and/or health of the animals.

## MATERIALS AND METHODS

### Location of the study area

The study was conducted in a commercial production unit situated in the municipality of Chihuahua, Chihuahua, in northern Mexico (28°29'N and 105°57'W). The Chihuahuan Desert, which is located at an elevation of 1440 meters above sea level and experiences an average temperature of 18.6 °C, is the location of the exploitation. This region receives rainfall between 150 and 500 mm during the summer-autumn season (INEGI, 2017; Schmidt, 1979).

The experiment was conducted during the months of October and November 2024, with the objective of mitigating thermal stress caused by elevated summer temperatures in the region and thereby isolating the effects of the treatments. The experiment involved 30 weaned Charolais calves, aged 180 days, which were divided into three homogeneous groups based on age and live weight. The base diet (DB) administered to the animals consisted of

corn stover, distiller's dry grain, rolled corn, urea, cane molasses, and probiotics derived from *Enterococcus faecium* and *Saccharomyces cerevisiae* (Probios® Precise, Chr. Hansen, Milwaukee, WI, USA) (Table 1). The following treatments were administered: The control group (T1) consisted of ten subjects with a mean body weight of  $154.05 \pm 3.26$  kilograms, who consumed the base diet at a rate of 3% of their body weight based on dry matter. The DBNN group (T2) included ten subjects with a mean body weight of  $155.79 \pm 2.26$  kilograms, who consumed the base diet plus 10% natural nopal. The DBNE group (T3) consisted of ten subjects with a mean body weight of  $156.75 \pm 3.10$  kilograms, who consumed the base diet plus 10% protein-enriched prickly pear cactus. Prior to the initiation of the experiment, all animals were vaccinated with 2 ml of Bobact® 8 (MSD Animal Health, Mexico City, Mexico), dewormed, and administered a vitamin with 2 ml of Ivermectin 2% + ADE (DIFESA, Guadalajara, Jalisco, Mexico).

The nutritional requirements of calves were calculated based on those proposed by the National Academies of Sciences, Engineering, and Medicine (NASEM) in 2016. The fodder cactus (*Opuntia ficus-indica*) utilized in the experimental diets was of the Atlxco variety. The protein enrichment of the nopal was achieved through a two-stage semi-solid fermentation process. Stage I:

In a plastic drum with a capacity of 200 liters, 40 liters of water (free of chlorine) are introduced, along with 1 kilogram of yeast (*Saccharomyces cereviceae*), 10 kilograms of cane sugar, and 10 kilograms of cane molasses. The mixture was stirred intermittently to facilitate oxygenation. Stage II of the process involved the use of specialized equipment to grind 1,000 kg of nopal and combine it with 10 liters of the mixture prepared in Stage I, along with 10 kilograms of urea and 1 kilogram of ammonium sulfate. The mixture was prepared by subjecting it to 30 minutes of constant agitation and subsequently allowing it to rest for an additional 30 minutes. Thereafter, the mixture was administered to the animals.

Table 1. Description and nutritional profile of the diets used in the preconditioning for export of Charolais calves in northern Mexico.

INGREDIENT	DB (%)	DBNN (%)	DBNE (%)
Enriched Nopal	-	-	10.09%
Natural Nopal	-	9.22	-
Corn stubble	25.50	23.15	22.95%
Rolled corn	39.98	36.29	35.98%
Distillery Dry Grain	28.53	25.90	25.68%
Urea	1.96	1.78	1.77%
Probiotics	0.10	0.10	-
Cane molasses	3.93	3.56	3.53%
<b>NUTRITIONAL PROFILE</b>			
Humidity (%)	49.54	44.41	43.93
Dry Matter (%)	50.46	55.59	56.07
Crude Protein (%)	19.57	18.25	20.19
Net Energy Gain (Mcal/kg)	1.27	1.24	1.22
Metabolizable Energy (Mcal/kg)	2.88	2.84	2.78
Total Digestible Nutrients (%)	79.63	78.53	77.03
Fat (%)	3.70	3.47	3.45
Ashes (%)	5.44	6.95	7.01
Neutral Detergent Fiber (%)	31.25	32.33	30.14

Calcium (%)	0.20	0.20	0.21
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DB= Base diet

DBNN=Base diet +10% natural nopal

DBNE=Base diet + 10% protein-enriched nopal.

The experiment spanned a duration of 45 days, encompassing an initial 15-day adaptation phase and a subsequent 30-day testing phase. The provision of sustenance occurred at two designated times per day (10:00 a.m. and 5:00 p.m., respectively). The provision of sustenance was conducted on an ad libitum basis, with the objective of ensuring a minimum consumption of 3% of the PV on a dry basis. Conversely, the quantity of food was recalibrated on a daily basis when the rejection rate was less than 5% of the total offered on the previous day. The animals were housed in group pens measuring 10 x 15 m to ensure a minimum of 15 m<sup>2</sup> of living space and 1 linear m of feeder per animal. Each pen was equipped with linear concrete feeders measuring 60 centimeters in width and 30 centimeters in depth. The animals had unrestricted access to clean, fresh water.

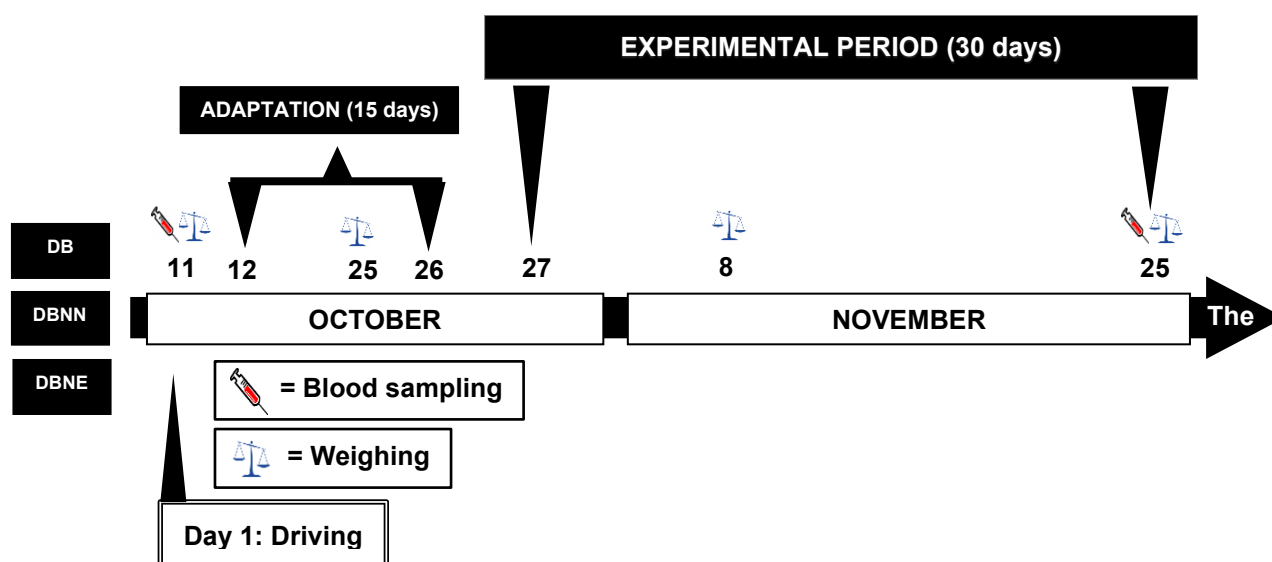


Figure 1. Experimental protocol for the inclusion of sources of forage prickly pear cactus in preconditioning diets for export for Charolais calves in northern Mexico. DB = Base diet; DBNN = base diet + natural prickly pear; DBNE = base diet + protein-enriched prickly pear cactus.

### Blood biometry

Blood samples were collected in 10-mL vacutainer tubes via jugular venipuncture. Blood samples were collected at the beginning and end of the study. The samples were collected on an empty stomach before serving food in the feeders, and were placed in a cooler to maintain a constant temperature until they were sent to the ASSAY clinical laboratory, located in the city of Chihuahua. The parameters that were the focus of the analysis included:

- White Formula:** leukocytes (uL), segmented neutrophils (%), lymphocytes (%), mixed cells (%), segmented neutrophils (uL), lymphocytes (uL) and mixed cells (uL).
- Red Formula:** erythrocytes (uL), hemoglobin (g/dL), hematocrit (%), mean corpuscular volume (MCV, fl), mean corpuscular hemoglobin (HCM, Pg) and mean hemoglobin concentration (MHC, g/dL).
- Platelets:** (uL) and cortisol (ug/dL).

The analysis of white blood cells, red blood cells, and platelets was performed by flow cytometry using an automated hematology analyzer (Sysmex KX-21N, Japan). While cortisol was analyzed by chemiluminescence in a cobas i 601 module (Roche Diagnostics, Switzerland).

### Ethical aspects

The study was carried out in accordance with the internal code of bioethics and taking into account both the animal welfare regulations of the FZyE of the UACH and the regulations of the Official Mexican Standard on animal care published by the Ministry of Agriculture and Rural Development (SAGARPA 1999).

### Statistical analysis

The information was analyzed through ANOVA under a mixed-effects model in a completely random arrangement. Starting weight was used as a covariate for final weight and daily weight gain. The comparison of means was performed through the Tukey test. The general structure of the model was:

$$Y_{ijkl} = \mu + B_{i(j)} + T_j + D_k + T_j * D_k + E_{ijkl}$$

Where  $Y_{ijkl}$  = is the dependent variable (PV, GDP, and/or hematological variables);  $\mu$  = constant that characterizes the population;  $B_{i(j)}$  = fixed effect of the nested  $i$ -th calf in the  $j$ -th treatment;  $T_j$  = fixed effect of the  $j$ -th treatment;  $D_k$  = random effect of the  $k$ -th day of testing;  $T_j * D_k$  = treatment interaction effect\* time; And  $E_{ijkl}$  = random error. All random components were assumed to be normally distributed, with mean zero and common variance. Similarly, the relationship between starting weight, ending weight, and daily weight gain was determined with a Pearson correlation analysis and partitioned by treatment to determine the direct effects of the diet. All analyses were performed with the SAS V9.3 statistical package.

## RESULTS

The results for the productive response are shown in (Table 2). No interaction effects were found, nor main effects between treatments ( $p > 0.05$ ) for final weight and daily weight gain.

Table 2. Productive response of Charolais calves in preconditioning for export fed with two sources of forage nopal in northern Mexico.

Variable	DB	DBNN	DBNE	P- Value	C.V.	R2
PI	154.05±3.26	155.79±2.26	156.75±3.10	0.2589	7.96	0.87
FAQ	178.74±3.91	177.97±2.72	172.99±3.72	0.5368	4.85	0.94
GDP	0.689±0.130	0.663±0.091	0.497±0.124	0.3072	46.30	0.28

DB= base diet; DBNN = base diet + natural prickly pear; DBNE= base diet + protein-enriched nopal; PI = initial weight; PF= final weight; GDP = daily weight gain; C.V. = coefficient of variation; R2 = coefficient of determination.

The behavior, in terms of daily weight gain, overtime, is shown in Figure 1. It is observed that at the beginning and end of the test the values are similar ( $p > 0.05$ ) between groups, however, in the middle of the test, a higher response ( $p < 0.05$ ) is observed in favor of the DB group.

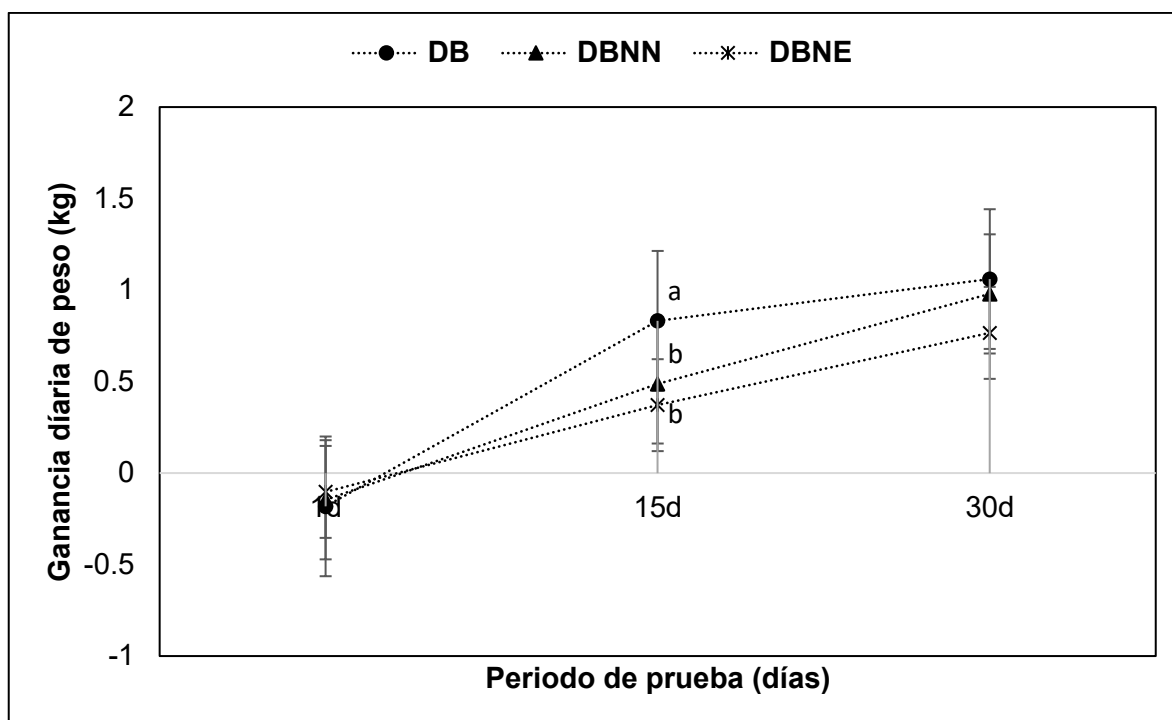


Figure 1. Daily weight gain in Charolai calves in preconditioning for export fed with two sources of forage nopal in northern Mexico. <sup>ab</sup>= different literals indicate statistical difference ( $p < 0.05$ ).

The ensuing section will present the relationship between productive variables due to treatments, as illustrated in Table 3. Positive and significant relationships were observed between PI and FP in the three treatments. In a similar vein, the DB and DBNN groups exhibited positive and significant relationships between PF and GDP. This finding is noteworthy, as the inclusion of prickly pear in the calves' diet has been shown to have a beneficial effect on their final weight. Nevertheless, in consideration of the brief evaluation period, the manifestation of the aforementioned effect was constrained.

Table 3. Correlation matrix for productive variables in Charolais calves in preconditioning for export fed with two types of nopal in northern Mexico.

	DB			DBNN			DBNE		
	PI	FAQ	GDP	PI	FAQ	GDP	PI	FAQ	GDP
PI	1.00			1.00			1.00		
FAQ	0.93***	1.00		0.85***	1.00		0.92***	1.00	
GDP	0.27	0.60*	1.00	0.14	0.65**	1.00	0.16	0.53	1.00

DB= base diet; DBNN = base diet + natural prickly pear; DBNE= base diet + protein-enriched nopal; PI = initial weight; PF= final weight; GDP = daily weight gain;  $=p < 0.0001$ ; \*\* $p < 0.05$ ; \* $=p < 0.10$ .

The results of the blood parameters are presented in (Tables 4 and 5). Differences between treatments were observed only on day 30 of the experiment, where the HCM and VAM parameters (Table 5) were higher in the DB group compared to the DBNN and DBNE groups.

Table 4. White formula of blood biometrics in Charolais calves in preconditioning for export fed with two sources of forage nopal, in northern Mexico.

Parameter	Sampling	DB	DBNN	DBNE	EE	P Value
Leukocytes, 103/ $\mu$ L	1, d	4.60	4.60	4.60	0.36	0.16
	30, d	6.00	7.10	6.78	0.36	0.30
Neutrophils, %	1, d	35.61	35.20	34.69	3.00	0.60
	30, d	27.75	24.87	26.70	3.00	0.75
Lymphocytes, %	1, d	49.67	48.48	49.03	2.55	0.79
	30, d	59.32	61.94	62.15	2.55	0.68
Mixed Cells, %	1, d	14.91	16.25	16.18	0.85	0.48
	30, d	13.11	13.12	11.05	0.85	0.68
Neutrophils, 103/ $\mu$ L	1, d	1.64	1.62	1.62	0.23	0.83
	30, d	1.69	1.81	1.93	0.23	0.62
Lymphocytes, 103/ $\mu$ L	1, d	2.26	2.27	2.26	0.20	0.06
	30, d	3.55	4.37	4.14	0.20	0.16
Mixed Cells, 103/ $\mu$ L	1, d	0.68	0.71	0.71	0.06	0.11
	30, d	0.75	0.92	0.72	0.06	0.99

DB= base diet; DBNN = base diet + natural prickly pear; DBNE= base diet + enriched prickly pear; EE= standard error; d = sampling day.

Table 5. Red formula of blood count, platelets and serum cortisol concentration in Charolais calves in pre-conditioning for export fed with two sources of forage nopal, in northern Mexico.

Parameter	Sampling	DB	DBNN	DBNE	EE	P Value
Erythrocytes, 106/ $\mu$ L	1, d	9.97	9.77	10.42	0.73	0.88
	30, d	9.11	9.10	7.21	0.73	0.34
Hemoglobin, g/dL	1, d	11.89	11.84	11.96	0.78	0.34
	30, d	10.3	11.91	10.80	0.78	0.73
Hematocrit, %	1, d	38.70	37.02	40.37	2.32	0.59
	30, d	34.40	33.17	32.28	1.60	0.93
VCM fl	1, d	38.76	38.75	38.62	0.47	0.67
	30, d	37.86a	37.48ab	36.10b	0.47	0.06
HCM Pg	1, d	11.82	11.83	11.80	0.15	0.17
	30, d	13.35a	12.89ab	12.45b	0.15	0.01
CMH g/dL	1, d	30.65	30.71	30.66	0.24	0.24
	30, d	35.25	34.56	34.71	0.24	0.30
Platelets, 105/ $\mu$ L	1, d	332	291	350	76	0.71
	30, d	546	645	590	76	0.70
Cortisol, ug/dL	1, d	2.07	2.12	2.3	0.52	0.75
	30, d	3.6	3.21	2.15	0.52	0.26

DB= base diet; DBNN = base diet + natural prickly pear; DBNE= base diet + enriched prickly pear; EE= standard error; d = day of sampling; MCV = mean corpuscular volume; HCM= mean corpuscular hemoglobin; HCM = mean hemoglobin concentration; <sup>ab</sup>=different literals indicate difference ( $p < 0.05$ ;  $p < 0.10$ ).

In addition to the productive response, a partial cost analysis was carried out to ascertain the economic feasibility of including natural nopal and/or protein-enriched nopal in diets for Charolais calves in preconditioning for export. The analysis encompassed the cost of rations (see Table 6), the quantity of feed offered, and the aggregate cost per day per animal.

The financial implications of these treatments were subsequently determined by calculating the cost per kilogram of the DB, DBNN, and DBNE treatments to be \$0.31, \$0.23, and \$0.22 USD, respectively. Concurrently, the average quantity of feed administered to animals per group, calculated based on dry matter, was 4.07 kilograms. Given that the duration of the experiment was 30 experimental days, the feeding cost for each of the groups was \$9.3, \$6.9, and \$6.6 USD per animal per day, respectively. In this regard, the incorporation of natural and/or protein-enriched nopal in the diets of calves prior to export results in a cost reduction of 26 to 29% compared to conventional diets. This information is of paramount importance, given that, although the production system in northern Mexico is centered on the export of calves, there has been a paucity of research on the optimization of feeding in the periods prior to the mobilization of livestock.

Table 6. Cost (USD) of the diets used in the preconditioning for export of Charolais calves in northern Mexico.

INGREDIENT (kg)	DB (%)		Cost	DBNN(%)	Cost	DBNE (%)	Cost
Enriched Nopal	-		\$-	-	\$-	10.09	\$0.39
Natural Nopal	-		\$-	9.22	\$0.32	-	\$-
Corn stubble	25.50		\$0.19	23.15	\$0.18	22.95	\$0.18
Rolled corn	39.98		\$0.66	36.29	\$0.58	35.98	\$0.58
Distillery Dry Grain	28.53		\$0.38	25.90	\$0.37	25.68	\$0.37
Urea	1.96		\$0.04	1.78	\$0.04	1.77	\$0.04
Probiotics	0.10		\$0.10	0.10	\$0.12	-	\$-
Cane molasses	3.93		\$0.14	3.56	\$0.10	3.53	\$0.10
**Cost (\$USD)/ kg	-		<b>\$0.31</b>	-	<b>\$0.23</b>	-	<b>\$0.22</b>

\*\*Average exchange rate for the year 2024 (<https://www.banxico.org.mx/>). 18.33 MXN= 1 USD.

## DISCUSSION

The productive response exhibited no significant disparities among the groups. In this regard, as previously stated, there is an absence of documented reports indicating the utilization of nopal in dietary interventions for the preconditioning of calves prior to export. However, the observed response aligns with the findings of previous studies conducted on other species of domestic ruminants, where no disparities were identified in PF and GDP in goats (Flores-Hernández et al., 2017) or lambs intended for fattening in the finishing stage (Da Trindade Silva et al., 2021).



Conversely, although no statistically significant differences were observed between the treatment groups, the control group exhibited a higher mean daily weight gain ( $p < 0.05$ ) at the midpoint of the experimental period. This phenomenon can be attributed to the observation that the inclusion of *Opuntia* in the diets of ruminants has been associated with extended periods of adaptation (Flores-Hernández et al., 2017). In this regard, Aguilar-Yáñez et al. (2011) reported that the digestibility of dry matter in lambs fed diets including *Opuntia* was lower at 6 and 12 hours, without differences at 24 hours. However, digestibility was higher in animals fed the cactus at 72 hours. Furthermore, Da Silva et al. (2022) reported that feeding time was prolonged in lambs fed diets with *Opuntia*, suggesting that this was due to a greater amount of water. This background corroborates the findings, as the calves in the DB group exhibited a greater weight gain in the middle of the experiment, yet by the conclusion of the study, they demonstrated similar weight outcomes across all groups.

Concurrently, all groups exhibited satisfactory growth under these management practices (Dhuyvetter et al., 2005), thereby substantiating the hypothesis that the incorporation of non-conventional ingredients, such as prickly pear forage, does not impact the productive response. In this regard, Nyambali et al. (2022) posit that the incorporation of 10% of *Opuntia* is a lucrative strategy to sustain animal production and ensure enhanced incomes in the face of escalating corn prices. In a similar vein, recent studies have demonstrated the viability of substituting an energy source, such as ground corn, with boneless cacti (*Opuntia ficus-indica*) in the diets of finishing lambs. This substitution has been shown to result in a substantial enhancement of the economic income for the producer, without any observed changes in daily weight gain, dry matter intake, consumption behavior, or yield of commercial cuts (De Alencar Alves et al., 2023).

Conversely, the provision of boneless cacti (*Nopalea cochenillifera* Salm-Dyck) to sheep has been linked to enhanced nutrient digestibility and elevated blood glucose levels (Maciel et al., 2019). These effects could explain the positive and significant relationships between GDP and FP in the DBNN group, in contrast to the trend observed in the DB group (Figure 1).

In the present study, all the blood values observed are within the normal range reported for the species (Motta et al., 2023; Roland et al., 2014). However, a lack of statistical significance was observed in the parameters of the white formula. Concurrently, Morshedy et al. (2020) documented no substantial disparities in red blood cells, white blood cells, or other blood parameters in lactating lambs of sheep fed diets augmented with prickly pear shells (*Opuntia ficus-indica*).

With regard to the red formula, which encompasses platelets and serum cortisol concentration, the only parameters that differed between groups were HCM ( $p < 0.05$ ) and VAW ( $p < 0.10$ ), both on day 30, at the conclusion of the experimental period. The DBNE group exhibited the lowest levels in both variables, without any significant differences observed between this group and the DBNN group ( $p > 0.05$ ). In this regard, it has been proposed that HCM and VAW could be associated with higher oxygen demand, an increase in basal metabolic rate, and less efficient animals in feed utilization (Cônsole et al., 2018).

This finding indicates that calves fed a diet deficient in nucleobases (DBNE) exhibited higher efficiency compared to calves fed a diet based on deoxyribonucleic acid (DNA). In this same sense, there are reports (Richardson et al., 2004) which suggest that stress stimulates energy mobilization in response to the stressing stimulus, causing cortisol levels to rise in animals with lower feed efficiency than that found in our study. The cortisol values in our study were not different between treatments; however, lower values were observed in calves fed DBNE compared to DB and DBNN. Conversely, the animals fed

DBNE exhibited reduced values of HCM and MCV, suggesting a diminished oxygen demand and, consequently, a reduced energy expenditure. This phenomenon may be linked to enhanced metabolic efficiency and reduced physiological stress (Chaves et al., 2015).

## CONCLUSION

The incorporation of forage prickly pear cactus (*Opuntia ficus-indica*) in preconditioning diets for export in Charolais calves does not affect productive behavior or animal health indicators. Furthermore, it results in savings of 26 and 29% compared to traditional diets. Consequently, it is determined that this approach constitutes a viable alternative for reducing production costs in northern Mexico.

Nevertheless, further comprehensive analyses of this substance on particular biomarkers of stress are advised. These analyses are intended to ascertain the full scope of its characteristics regarding production efficiency and animal welfare.

## ACKNOWLEDGEMENTS

The authors thank Mr. Luis Fernando Prieto Astill and Dr. José Raúl Reyes Roel, for the funding and their invaluable support during the preparation of this work.

## DECLARATION OF CONFLICT OF INTEREST OF AUTHORS

The authors declare that they have no conflict of interest.

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