



# Effects of Cactus Flour (*Opuntia ficus-indica*) on Productive Performance and Eggshell Quality of Laying Hens

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## ABSTRACT

The poultry industry plays a crucial role in the production of animal proteins for human consumption and generating sources of employment. Thus, it is essential to explore effective strategies to enhance both the productivity of laying hens and the quality of their products, particularly eggshell quality, due to its significant economic implications for the poultry sector. This study aimed to evaluate the effects of cactus flour (CF; *Opuntia ficus-indica*) on the productive performance and eggshell quality of laying hens. Twenty-four Rhode Island Red laying hens were randomly divided into two groups (n = 12 experimental units -hens-/group) consisting of a control group and another fed CF (1% of the diet volume). The variables assessed included initial and final weight, weight gain, feed and calcium intake, egg production, egg mass, feed conversion index/kg of egg, economic efficiency index, egg weight, shell weight, shell thickness, shell percentage, and calcium levels in eggshells and excreta (daytime and nighttime). The addition of CF in the diet affected the final weight of hens, with the CF-fed hens (2.1 kg) being heavier than the control (1.9 kg). Egg production was higher in the CF-fed hens than in the control hens. Additionally, the mean egg weight was higher (68.5 g) in the CF-fed hens than in the control (62.2 g). The feed conversion index was lower in the CF-fed hens (2.1 kg/kg) than in the control (1.8 kg/kg). The economic efficiency index was higher in the CF-fed hens (94.8 %) than in the control (90.2 %). Eggshell weight (5.8 g), thickness (0.31 mm), and calcium levels (35.6 %) were significantly higher in the CF-fed hens than in the control (5.1 g, 0.27 mm, and 33.4 % for eggshell weight, thickness, and calcium levels, respectively). In conclusion, the inclusion of CF in the diet of laying hens improved the productive indicators and eggshell quality, thereby enhancing economic efficiency.

**Keywords:** Egg production, Cactus flour, Calcium, Laying hens, Poultry farming

## INTRODUCTION

Genetic improvements have increased the productivity of laying hens and caused physiological modifications, which can lead to productive inefficiency in these animals (Li et al., 2017). Therefore, further research is important to establish efficient strategies to improve productivity indicators in hens (Geraldo et al., 2006). Specifically, eggshell quality is the most important indicator associated with the improved productive performance of laying hens. It has important economic repercussions on egg production, accounting for 6-10% of the total decrease in

egg production (Lichovnikova, 2007; Świątkiewicz et al., 2015; An et al., 2016).

Since calcium carbonate makes up 95% of the eggshell's chemical composition, calcium supply is the most important nutritional factor for eggshell quality and productive longevity as hens (Li et al., 2017). With increased longevity, hens produce eggs with weak shells and compromised skeletal structure due to the decreased efficiency of the intestinal absorption of calcium (Diana et al., 2021), leading to greater dependence on bone-derived calcium, thus increasing the risk of fractures (de Juan et al., 2023). The National Research Council (NRC, 1994)

prescribed the mineral requirements for poultry almost 30 years ago. Since then, the genetic makeup, housing, management, and diet of laying hens have changed (Li et al., 2017).

Many studies have reported discrepancies in the calcium requirements for laying hens established by the NRC (1994). Previous studies have demonstrated that the NRC-specified calcium levels are adequate for optimal shell formation and that an additional increase in the dietary calcium level (> 3.6%) does not affect the eggshell quality (Valkonen et al., 2010; Pastore et al., 2012). However, variations in eggshell quality have also been reported using the calcium levels outlined by NRC (1994). This may be due to the variable absorption of calcium. Therefore, source and bioavailability of minerals, serum levels of calcium, phosphorus, vitamin D3, parathyroid hormone, gastrointestinal pH, and mineral content in the diet should be evaluated as indicators of dietary calcium usage (Koreleski and Swiakiewicz, 2004; Lichovnikova, 2007; Li et al., 2017; de Juan et al., 2023).

Determining the optimal calcium requirements for laying hens is a challenge for nutritionists and poultry producers (Pastore et al., 2012; An et al., 2016). Continuous genetic improvements in commercial hens, without considering the modifications of their nutritional requirements, along with the increasing incorporation of novel ingredients in animal diets to reduce reliance on conventional raw materials, have expanded research on alternative ingredients for animal feed (Andhale, 2024). In women, the consumption of cactus flour (CF) improves osteoporosis by counteracting the reduction in bone mineral density due to menopause; this effect is attributed to the amount (35.3 mg/g) and bioavailability (0.12 molar ratio of oxalate; Calcium) of calcium in CF (Aguilera-Barreiro et al., 2013; Rojas-Molina et al., 2015).

The consumption of CF by breeding sows (Ordaz et al., 2020) and rats (Kang et al., 2012) increases the concentration of osteocalcin, a marker of bone formation associated with improved bone mineral density, possibly due to the high calcium content of CF (Hernandez-Becerra et al., 2020). According to these reports, adding CF to the diet could improve the eggshell quality-related indicators in laying hens. It should be noted that the results of the use of CF in laying hens have been limited to the use of CF from cacti older than two years (Sousa et al., 2024). These are characterized by being metabolically unviable for monogastric, including humans, due to their high lignin content (Castellano et al., 2021). Therefore, the current study aimed to assess the impact of CF (*O. ficus-indica*) on the productivity and eggshell quality of laying hens.

## MATERIALS AND METHODS

### Ethical approval

The study adhered to the Official Mexican Standard 062-ZOO (NOM-062-ZOO, 1999) for the production care and use of laboratory animals, and the technical specifications of the International Guiding Principles for Biomedical Research with Animals for the Council for International Organizations of Medical Sciences (CIOMS, 1985) in the handling of all the hens.

### Experimental conditions

The study was performed during May and June 2023 at the poultry facilities of the Faculty of Veterinary Medicine and Zootechnics (FVMZ) of the Universidad Michoacana de San Nicolás de Hidalgo (UMSNH), Mexico. The FVMZ-UMSNH is located at km 9.5 of the Morelia Zinapécuaro highway, municipality of Tarímbaro, Michoacán, between the coordinates 19°47'11" north latitude and 101°10'35" west longitude, at an altitude of 1,864 meters above sea level. The climate is temperate subhumid with summer rains, with annual rainfall ranging from 600 to 800 mm and temperatures between 2.5 and 25.1°C (INEGI, 2017).

### Animals, diet, and housing

In the present study, twenty-four Rhode Island Red hens, aged 75 weeks, with 53 weeks of laying experience, were employed. Each hen was considered an experimental unit. Every hen was allocated at random to a single, standard battery-style cage measuring 46 × 40 × 43 cm in length, width, and height. The cages were equipped with automatic waterers and galvanized iron feeders that covered the entire front region of the cage. The hens were randomly divided into two groups (12 hens - experimental units-/group) that included hens that did not consume CF (control) and animals that consumed CF 1% of the volume of the diet ration (Experimental; Table 1). The diets were balanced according to the recommended requirements of the National Research Council (NRC, 1994) for laying hens. The hens were administered the diets for 10 weeks, followed by two weeks of adaptation and 8 weeks of evaluation. Notably, 120 g/hen/day feed was supplied to both groups. Free access to water was provided.

### Obtention and production of cactus pear meal

The cladodes used to produce the flour weighed 500 ± 50 g (approximately 125 days old). For dehydration, the cladodes were cut into 10 cm long and 1 cm wide fragments and dried in the sun on metal trays for a week.

They were subsequently ground using a 0.5-mm mesh in a laboratory mill (Arthur H. Thomas Co. Philadelphia, PA).

**Table 1.** Composition and nutritional value of the diet with cactus flour for laying hens

Ingredients (%)	Control diet	Cactus flour diet
Corn	69.00	68.00
Soybean meal	18.88	18.88
Limestone	8.55	8.55
Cactus flour	--	1.00
Dicalcium phosphate	1.35	1.35
Kaolin	0.59	0.59
Soy oil	0.53	0.53
Minerals + vitamins premix <sup>&amp;</sup>	0.50	0.5
Salt (NaCl)	0.43	0.43
DL-methionine-98%	0.12	0.12
L-lysine-78%	0.03	0.03
Butylhydroxytoluene	0.02	0.02
Total	100.00	100.00
<b>Estimated analysis</b>		
Metabolizable energy (kcal/kg)	2800	2800
Crude protein (%)	15.00	15.37
Crude fiber (%)	4.00	4.10
Total calcium (%)	3.60	4.80
Total phosphorus (%)	0.40	0.41
Digestible arginine (%)	0.84	0.84
Digestible lysine (%)	0.64	0.64
Digestible methionine (%)	0.34	0.34
Digestible Met + Cis (%)	0.56	0.56

<sup>&</sup>Levels per Kg of diet: Vit. A – 8000 IU; Vit. D3 – 2000 IU; Vit. E – 50 mg; Vit. K – 3 mg; Vit. B1 – 1.5 mg; Vit. B2 – 4 mg; Vit. B6 – 0.12 mg; Vit. B12 – 15 mg; Ac. Folic – 0.6 mg; Ac. Pantothenic – 10 mg; Niacin – 30 mg; Biotin – 0.1 mg; Choline – 300 mg; Iron – 50 mg; Copper – 10 mg; Zinc – 70 mg; Manganese – 100 mg; Iodine – 1 mg; Selenium – 0.3 mg; Antioxidants 50 mg.

### Laboratory analysis

The chemical composition of CF (Table 2) used in the diet was analyzed by the FVMZ-UMSNH Nutrition and Food Analysis Laboratory. An adiabatic bomb calorimeter (Model 1281; Parr, Moline, IL) was used to measure the energy content of raw materials, experimental diets, and CF. The dry matter and crude protein contents were determined following the AOAC (2000) guidelines 934.01 and 976.05, respectively. The Mexican Standard-Y-021-SCFI (NMX-Y-021-SCFI, 2003) was used to measure the calcium levels in CF and experimental diets.

**Table 2.** Chemical composition of cactus flour (*O. ficus indica*)

Indicator	Amount
Humidity (%)	7.33
Protein (%)	12.87
Fat (g)	2.53
Soluble fiber (g)	14.91
Insoluble fiber (g)	41.65
Ash (%)	21.17
Calcium (%)	4.33
Phosphorus (%)	0.29
Calcium oxalates (mg/g)	5.73
Calcium/Phosphorus Ratio	14.93

### Experimental procedure

The body weight of each hen was recorded at the beginning and end of the experimental period using a digital scale (Dibatec<sup>®</sup>; 0.005–40 kg). Feed intake was assessed based on the difference with the supply (120 g) using a digital scale (Noval NBE-CF<sup>®</sup>; 0.01–2 kg). Egg production was determined by the Formula 1.

$$\text{Egg production (\%/hen/day)} = \left( \frac{\text{number of eggs laid}}{\text{number of hens}} \right) * 100 \quad (\text{Formula 1})$$

Egg weight (g), which was calculated using a digital scale (OHAUS Scoutv<sup>®</sup>; 120 g x 0.001 g).

Egg mass was determined by the Formula 2.

$$\text{Egg mass (g/hen/day)} = \text{egg weight} * \text{egg production} \quad (\text{Formula 2})$$

The feed conversion ratio was determined by the Formula 3.

$$\text{Feed conversion index (FCI) (kg/kg)} = \left( \frac{\text{feed intake}}{\text{egg mass}} \right) \quad (\text{Formula 3})$$

Calcium intake (g/hen/day), which was estimated according to the feed intake and calcium that the diets contained, shell weight (g), which was calculated using a digital scale (OHAUS Scout®; 120 g x 0.001 g), shell thickness (mm), which was estimated using a digital micrometer (Baxlo®; 0–10 mm),

Shell percentage was determined by the Formula 4.

$$\text{Shell percentage} = \left( \frac{\text{shell weight}}{\text{egg weight}} \right) * 100 \quad (\text{Formula 4})$$

Shell calcium (%), which was determined from a mineral solution through atomic absorption spectrometry, excreted calcium (%) at daytime (18 hours) and excreted calcium at nighttime (7 hours), which were determined based on the calcium intake and the calcium determined in the feces, and the economic efficiency index (EEI %).

To determine the economic viability of adding CF to the diet, the cost of the diet per kg of eggs produced ( $Y_i$ ) was determined using the equation adapted from the methodology of Bellaver et al. (1985; Formula 5).

$$Y_i = \frac{P_i * Q_i}{E_i} \quad (\text{Formula 5})$$

where  $Y_i$  is the diet cost per kg of eggs produced in the  $i^{\text{th}}$  treatment (control, HN),  $P_i$  is the price per kg of the diet used in the  $i^{\text{th}}$  treatment,  $Q_i$  is the quantity of diet consumed in the  $i^{\text{th}}$  treatment, and  $E_i$  is the kg of eggs produced. The economic efficiency index (EEI) was determined using the following equation (Formula 6).

$$EEI = \left( \frac{LCe}{CTei} \right) \times 100 \quad (\text{Formula 6})$$

where LCe is the lowest diet cost per kg of eggs produced with different treatments, and CT<sub>ei</sub> is the cost of the  $i^{\text{th}}$  treatment.

### Statistical analyses

All statistical analyses were conducted using SAS version 9.4, 2020 (SAS Institute Inc, Cary, NC, USA). Before data analysis, the normality of the distribution and homogeneity of variance for the residuals were determined using PROC UNIVARIATE. The Shapiro–Wilk test was used to determine normality, whereas the Bartlett test was used to determine homogeneity. The data were analyzed using a completely randomized design with PROC GLM (model determined by likelihood ratio test). The hen was taken as the experimental unit. The model included diet as a fixed effect and hen as a random effect for statistical

analysis. Correlations between feed intake and calcium consumption and the variables associated with productive performance and economic efficiency, egg quality, and calcium kinetics were analyzed using the Pearson correlation test (PROC CORR). Differences between means were determined using the least squares means method with  $p\text{-value} \leq 0.05$ . The values were represented as least squares mean  $\pm$  standard error of the mean.

## RESULTS

Data analysis revealed a diet effect ( $p < 0.001$ ), with the CF-fed hens exhibiting a significantly higher weight at the end of the experiment compared to the control group (Table 3). This increase in weight was attributed to a significant weight gain ( $p < 0.05$ ), with CF-fed hens gaining an average of 49.7 g more than the control (Table 3). Egg production and mass were significantly higher in the CF-fed hens ( $p < 0.05$ ; 88.6 %/hen/day and 60.1% g/hen/day, respectively) compared with the control (85.7 %/hen/day and 52.9 g/hen/day for egg production and mass, respectively). Higher egg production resulted in significantly lower ( $p < 0.05$ ) FCI (1.8 kg/kg) and higher ( $p < 0.05$ ) EEI (94.8%) in the CF-fed hens compared to the control group (2.1 kg/kg and 90.2% for FCI and EEI, respectively; Table 3).

Further diet analysis revealed that the CF-fed hens exhibited significantly higher egg weight ( $p < 0.05$ ; 68.5 vs. 62.2 g), shell weight (5.8 vs. 5.1 g), and calcium levels in the shell (35.6 vs. 33.4%) than the hens in the control group (Table 4). Evaluation of the diets revealed that CF significantly increased the calcium consumption of hens ( $p < 0.05$ ; 5.4 g/day) compared to other experimental diets (4.1 g/day; Table 5). Calcium levels in daytime excreta were significantly higher in the hens that did not consume CF ( $p < 0.05$ ; 7.6 vs. 5.7%); however, no significant differences in the calcium levels in nocturnal excreta were observed between the groups ( $p > 0.05$ ; Table 5).

The results also indicated that the feed and calcium intake were differently associated with variables affecting productive and economic efficiency, egg quality, and calcium kinetics. For the variables affecting productive and economic efficiency, feed intake was significantly associated with weight gain in both the control and the CF-fed hens ( $r = 0.34$  and  $0.52$ ;  $p < 0.05$ ; Figure 1a). Calcium intake was also significantly associated with egg production (egg mass) in both the control ( $r = 0.64$ ;  $p < 0.05$ ) and the CF-fed hens ( $r = 0.53$ ;  $p < 0.05$ ). Similarly, egg production (egg mass) was significantly associated ( $p < 0.05$ ) with both FCI and EEI, showing correlations

greater than 0.80 in both groups. However, the correlation between egg mass and feed conversion ratio was negative in both groups (Figure 1a). Next, correlations between variables affecting egg quality and feed and calcium intake were analyzed (Figure 1b). Feed intake was significantly associated with egg weight and shell thickness in the control and the CF-fed hens ( $r \geq 64$ ;  $p < 0.05$ ; Figure 1b). Calcium intake was also significantly associated ( $p < 0.05$ ) with the shell thickness in both the control and the CF-fed hens ( $r = 0.59$  and  $0.67$ , respectively). Additionally, egg

production was significantly associated with egg weight ( $r \geq 72$ ;  $p < 0.05$ ) in both groups. In the control group, feed intake was not significantly associated ( $p > 0.05$ ) with any variable determining calcium mobilization (Figure 1c). In the CF-fed hens, feed intake was associated with shell percentage ( $r = 0.40$ ;  $p < 0.05$ ) and shell calcium ( $r = 0.37$ ;  $p < 0.05$ ). The CF-fed hens exhibited stronger associations ( $p < 0.05$ ) of calcium levels in the shell with calcium levels in the daytime and nighttime excreta than the hens that did not consume CF (Figure 1c).

**Table 3.** Effect of cactus flour intake on productive indicators in laying hens (mean  $\pm$  SD)

Indicator	Control diet	Cactus flour diet	P - value
Initial body weight (kg)	1.817 $\pm$ 0.19	1.952 $\pm$ 0.21	0.1507
Final body weight (kg)	1.887 $\pm$ 0.18 <sup>a</sup>	2.072 $\pm$ 0.16 <sup>b</sup>	< 0.0001
Weight gain (g)	70.3 $\pm$ 3.28 <sup>a</sup>	120.0 $\pm$ 4.11 <sup>b</sup>	0.0104
Feed intake (g/hen/day)	112.9 $\pm$ 6.23	112.6 $\pm$ 5.33	0.8459
Production (%/hen/day)	85.7 $\pm$ 2.04 <sup>a</sup>	88.6 $\pm$ 2.46 <sup>b</sup>	0.0019
Egg mass (g/hen/day)	52.9 $\pm$ 6.29 <sup>a</sup>	60.1 $\pm$ 5.09 <sup>b</sup>	0.0123
Feed conversion rate (kg/kg)	2.1 $\pm$ 0.19 <sup>b</sup>	1.8 $\pm$ 0.09 <sup>a</sup>	0.0062
Economic efficiency index (%)	90.2 $\pm$ 1.76 <sup>a</sup>	94.8 $\pm$ 2.17 <sup>b</sup>	0.0018

<sup>a-b</sup>Means in the same row with superscripts differ significantly ( $p < 0.05$ ).

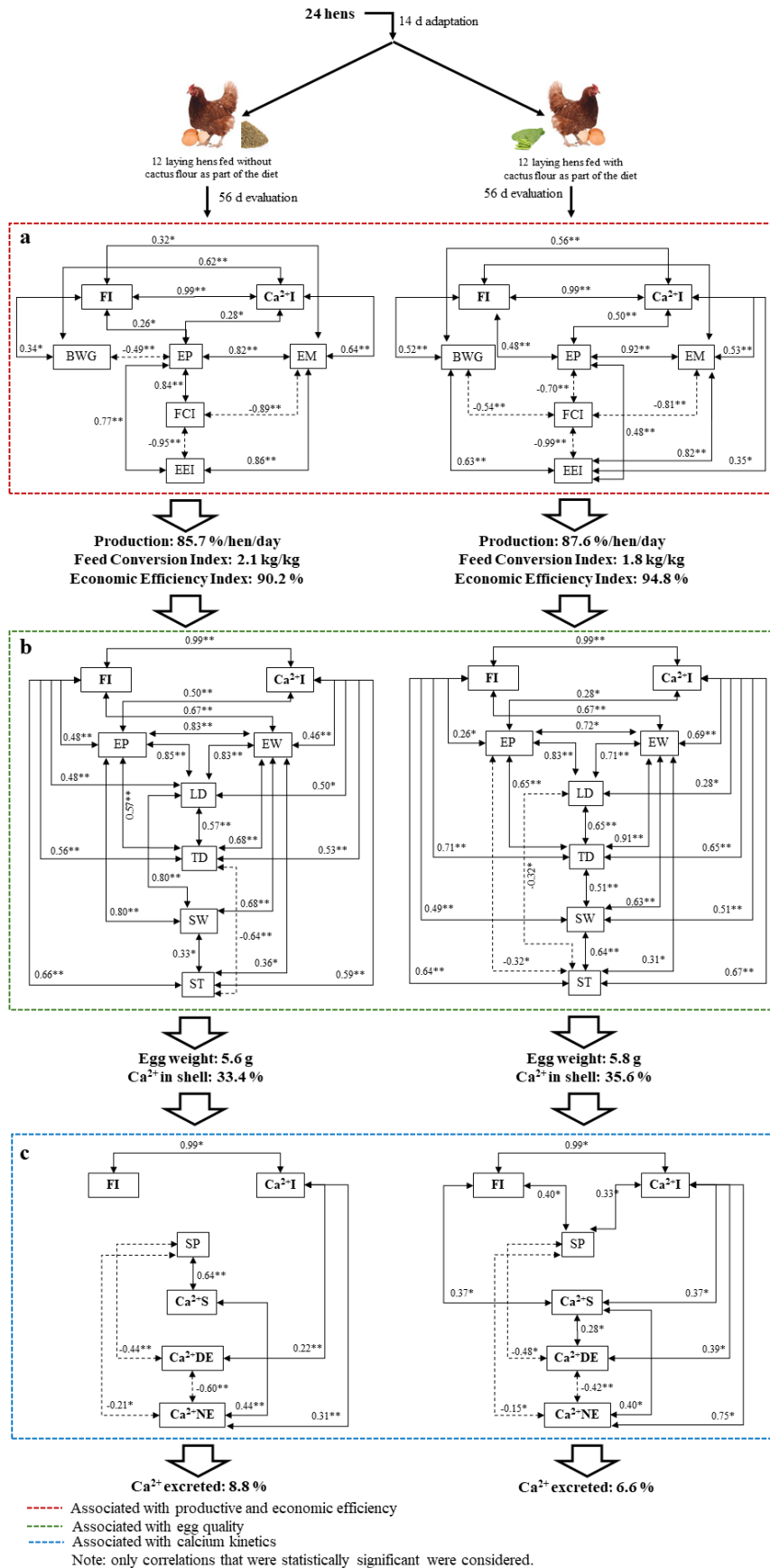
**Table 4.** Effect of cactus flour intake on egg quality indicators in laying hens (mean  $\pm$  SD)

Indicator	Dieta control	Cactus flour diet	P - value
Egg weight (g)	62.2 $\pm$ 6.2 <sup>a</sup>	68.5 $\pm$ 4.09 <sup>b</sup>	0.0493
Shell weight (g)	5.1 $\pm$ 0.70 <sup>a</sup>	5.8 $\pm$ 0.31 <sup>b</sup>	< 0.001
Longitudinal diameter (cm)	6.0 $\pm$ 0.14 <sup>a</sup>	6.1 $\pm$ 0.17 <sup>b</sup>	0.0580
Transverse diameter (cm)	4.3 $\pm$ 0.09	4.4 $\pm$ 0.15	0.3509
Shell thickness (mm)	0.27 $\pm$ 0.006 <sup>a</sup>	0.31 $\pm$ 0.003 <sup>b</sup>	0.0019
Shell percentage (%)	8.2 $\pm$ 0.83 <sup>a</sup>	8.5 $\pm$ 0.40 <sup>b</sup>	0.0431
Calcium in shell (%)	33.4 $\pm$ 2.52 <sup>a</sup>	35.6 $\pm$ 1.44 <sup>b</sup>	< 0.001

<sup>a-b</sup>Means in the same row with superscripts differ significantly ( $p < 0.05$ ).

**Table 5.** Effect of cactus flour intake on indicators of calcium mobilization in laying hens (mean  $\pm$  SD)

Indicator	Dieta control	Cactus flour diet	p-value
Calcium consumption (g/hen/day)	4.1 $\pm$ 0.21 <sup>a</sup>	5.4 $\pm$ 0.55 <sup>b</sup>	< 0.001
Calcium in diurnal excreta (%)	7.6 $\pm$ 1.17 <sup>a</sup>	5.7 $\pm$ 0.70 <sup>b</sup>	< 0.001
Calcium in nocturnal excreta (%)	10.0 $\pm$ 0.57	9.6 $\pm$ 0.76	0.2962



**Figure 1.** Schematic representation of the Pearson correlation coefficients for feed intake and calcium intake in laying hens fed with cactus flour with variables that affect productive and economic efficiency (a), egg quality (b), and calcium mobilization (c). FI: Feed intake, Ca<sup>2+</sup>I: Calcium intake, BWB: Body weight balance, EP: Egg production, EM: Egg mass, FCI: Feed conversion index, EEI: Economic efficiency index, EW: Egg weight, LD: Longitudinal diameter of the egg, TD: Transverse diameter of the egg, SW: Shell weight, ST: Shell thickness, SP: Shell percentage, Ca<sup>2+</sup>S: Calcium in shell, Ca<sup>2+</sup>DE: Calcium in diurnal excretion, Ca<sup>2+</sup>NE: Calcium in nocturnal excretion. \*: Significant (p < 0.05); \*\*: Highly significant (p < 0.001).

## DISCUSSION

Calcium is a vital nutrient for laying hens, especially for bone formation and as an enzyme cofactor (Li et al., 2016). Calcium is essential for blood coagulation, eggshell formation, and muscle and nerve function (Li et al., 2017). However, the impact of dietary calcium levels on laying hen's productivity remains a topic of debate. Some studies suggested hens can produce adequate eggs and operate productively with as little as 3.2% calcium in their diet (Świątkiewicz et al., 2015). According to Cufadar et al. (2011), adding 3.0, 3.6, or 4.2% of calcium to the diet did not significantly affect egg production, egg weight, egg mass, or feed conversion index (FCI). Safaa et al. (2008) plotted the egg production curve and reported that hens require more than 3.5% calcium in their diet for optimal laying performance and that increasing the level of dietary calcium to 4.0% improved egg production, egg mass, and FCI.

Cactus flour (CF) is considered a good source of minerals, including high concentrations of calcium, potassium, magnesium, and phosphorus (Santana et al., 2021). Sousa et al. (2024) reported that the addition of 3.0, 6.0, and 9.0% CF to the diet did not affect the productive variables of laying hens. The authors attributed these findings to the presence of calcium oxalate, an anti-nutritional factor in CF that binds to calcium, making it unavailable, thus impacting the bioavailability of calcium for animal absorption (McConn and Nakata, 2004; Batista et al., 2009). Notably, older cactus plants tend to have lower concentrations of calcium oxalate (Rodríguez-García et al., 2007). Sousa et al. (2024) observed no effect of CF intake on the productivity of laying hens when cladodes aged two years were used likely due to a decrease in phosphorus content as cladodes mature, which alters the calcium/phosphorus ratio and subsequently reduced the bioavailability of calcium (Rodríguez-García et al., 2007; Hernández-Urbiola et al., 2010). Furthermore, as the cladode ages, the levels of cellulose, hemicellulose, and lignin increase, compromising the digestibility of nutrients, as the gut does not synthesize the enzymes needed for their degradation (Castellano et al., 2021).

Regarding egg quality variables, such as shell thickness and percentage, and productive variables, many studies have reported contradictory results using the calcium levels outlined by NRC (1994). For instance, one study found that laying hens fed diets with high concentrations of calcium (4.4%) exhibited reduced shell thickness than the hens in the control group (3.7%

calcium; Jiang et al., 2013). However, in another study, eggshell quality (shell weight and thickness) improved when the dietary calcium level was increased from 3.5 to 4.0% (Safaa et al., 2008). Studies using laying hens between 58 and 93 weeks of age have reported higher requirements of calcium for optimal eggshell quality than those outlined by the NRC (1994).

In the present study, enhanced eggshell quality in CF-fed hens was associated with the quantity and bioavailability of minerals (calcium, phosphorus, and magnesium) found in nopal, which aligns with previous reports (Hernández-Urbiola et al., 2010; Rojas-Molina et al., 2015; Quintero-García et al., 2020). The bioavailability of calcium was closely associated with the phosphorus content of the diet. Owing to the low availability of phosphorus and its antagonism with calcium, increasing the concentration of calcium without considering the phosphorus levels can adversely affect the egg quality and health in hens, increasing the risk of fractures (Sinclair-Black et al., 2023). The total calcium/phosphorus ratio in the diet should be 2:1 for all species; however, in laying hens, this ratio can be increased up to > 7:1. In terms of available phosphorus, this ratio (calcium/available phosphorus) can reach up to 10-12:1 (de Araújo et al., 2015). Here, the calcium/phosphorus ratio in the CF versus control diet was 11.7 vs. 9.0, thus leading to greater bioavailability of calcium in the CF-fed hens. Furthermore, the molar ratio of oxalate/calcium according to age was approximately 0.12, indicating that molar ratios of oxalate/calcium  $\geq 1.0$  indicated a lack of calcium availability (Bhandari and Kawabata, 2004; Rojas-Molina et al., 2015). Therefore, based on the oxalate/calcium ratio, the bioavailability of calcium in the CF-fed group was not compromised.

Increased bioavailability of calcium in the CF-containing diet possibly influenced bone matrix formation, thereby minimizing the use of endogenous calcium and enhancing the eggshell quality. Changes in eggshell quality showed correlations between calcium consumption and eggshell weight and thickness, with stronger correlations observed in the CF-fed hens than in the control. Similarly, higher intake and bioavailability of calcium in the CF diet resulted in higher calcium levels in the shell (higher weight, thickness, and calcium percentage in the shell) and lower calcium levels in the excreta compared to those in the control group (6.6 vs. 8.8%). These results showed that the CF-fed hens exhibited stronger correlations between calcium intake and calcium



levels in the shell and calcium levels in the daytime and nighttime excreta compared to controls.

## CONCLUSION

The results indicated that the inclusion of cactus flour in the diet of laying hens enhanced productivity and eggshell quality. The best egg quality and feed conversion rate per kilogram of produced eggs were obtained at 1% CF, which also improved the economic efficiency index (EEI) per kilogram of produced eggs. Nevertheless, further research is needed to determine the optimal level of CF in the diets of laying hens.

## DECLARATIONS

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### Availability of data and materials

The data that support the findings of this study are available on request from the corresponding author.

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### Authors' contributions

AJ and GO designed the experiment, EG and CV conducted the experiment, AJ, and GO completed data analysis; AJ supplied resources, AJ and GO wrote the original draft manuscript, AJ and EG corrected the manuscript, AJ and CV supervised the project. All authors have checked the collected, and analyzed data and agreed on the submission of this article.

### Competing interests

The authors assert that they have no competing interests.

### Ethical considerations

All authors have reviewed ethical concerns, including data fabrication, double publication and submission, redundancy, plagiarism, consent to publish, and misconduct before being published in this journal.

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