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Article

Use of Cactus Pear Meal in the Feeding of Laying Hens in Semi-Intensive System

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Simple Summary: This study explores the effectiveness of use of cactus pear meal in the feeding of laying hens to improve production and reduce costs. The objective was to evaluate the impact of different proportions of this flour on the birds' diet. The variety "Miúda" of cactus pear was found to be the most efficient, improving energy utilization and nutrient digestibility. Although the birds' productive performance was not significantly affected with the use of up to 9% of flour in the diet, improvements were observed in egg quality, including texture and color of the yolk, as well as a healthier composition in terms of fatty acids and cholesterol. The study concludes that the inclusion of 3% cactus pear meal is the most economically suitable, offering benefits without compromising quality or performance. These findings are important for egg farmers who seek sustainable and economical alternatives, thus contributing to a more conscious society regarding food production and nutrition.

Abstract: Little information is available in the literature on the use of cactus pear meal in poultry diets being important to evaluate diets that provide excellent performance and lower production costs. Our objective was to study the use of cactus pear meal in diets of laying hens. In the first study, two diets for male and female chicks were used - 1: 80% reference diet + 20% *Miúda* cactus pear meal (CPM) and 2: 80% reference diet + 20% *Gigante* cactus pear meal. The variety *Miúda* provided better use of metabolizable energy, digestibility coefficient of dry matter, protein, and mineral matter. In the second study, a control diet was compared to three diets with different levels of CPM for laying hens, in the proportions of 3%, 6% and 9%. No significant differences were found in productive performance. However, there were significant differences in egg quality, texture and color profile of the cooked yolk, egg composition, fatty acids and cholesterol in the yolk. It is possible to use 9% of CPM in the diet of laying hens in semi-intensive system not compromising performance and egg quality, and using 3% of CPM provides higher economic return.

Keywords: Alternative feed; productive performance; laying; egg quality

1. Introduction

Due to their low cost and great nutritional profile, eggs are considered a highly nutritive food [1] that is consumed daily. Brazilian egg production has been rapidly growing, in 2021 it totaled 54.9 billion per unit and consumption per person reached 277 units per person [2]. Changes in the consumer eating patterns and rising prices of other proteins have contributed to the that increase.

Consumers show preference for poultry products from alternative systems (cage-free, free-range, organic, and rustic free-range), with stricter safety rules and welfare concepts [3].

Therefore, large companies became aware of market innovations and have adopted alternative egg production systems, which has led to diversification of egg categories available on the market [4]. The semi-intensive system consists of a production model in which hens have access outdoor areas, where they are released in the morning and gathered in the afternoon, which can positively impact production performance and egg quality.

Increasing prices of commodities used in poultry feeding has had significant impact on the poultry industry, affecting production costs. Corn, which is the main energy source for poultry farming, is increasingly disputed by the food industry, making it unaffordable and expensive, with increases of about 200% from 2015 to 2021 [2]. Therefore, one way to reduce the cost of feed is to seek alternative sources to corn that are effective, accessible, inexpensive, and suitable for the region.

Cactus pear is a widely used alternative animal feed in the Brazilian Northeast because it is considered an energy-source feed due to the high concentration of non-fiber carbohydrates [5–7]. It also presents good adaptability characteristics and high productive potential to the edaphoclimatic conditions of this region [8,9], besides the wide availability which reaches approximately 600,000 ha cultivated in Brazil. The most widely used genotypes are *Opuntia* with cultivars *Gigante* and *Redonda* and *Nopalea* with cultivar *Miúda* [10]. Among the cactus pear cultivars, *Miúda* (*Nopalea cochinilifera* (L.) Salm Dyck) stands out for its nutritional qualities: high contents of total carbohydrates (822.1 g/kg), non-fiber carbohydrates (597.5 g/kg), mineral matter (128.8 g/kg) and low contents of neutral detergent fiber (224.6 g/kg) and acid detergent fiber (189.7 g/kg) [6], in addition, it presents high energy content (3,653 kcal/g) [11].

Among the forms of supply, *Miúda* cactus pear meal (CPM) which is a product obtained after dehydration of the cladodes, contains 82.2% dry matter (DM), 8% crude protein (CP), 1% ether extract (EE), 25.1% neutral detergent fiber (NDF), 46.7% non-fiber carbohydrates (NFC), 18.5% mineral matter (MM), 2.3% calcium, 0.2% phosphorus [12] and 3,647 kcal/g gross energy (GE) [11], with great potential to be a cheap and accessible energy source.

Some available studies on cactus pear meal show promise in the feeding of broilers in free-range system [11], industrial broiler [13] and quails [14]. As far as it is known, there is no published study on the effect of cactus pear meal in the feeding of laying hens, and research is needed to demonstrate its potential and to measure its value. Thus, the objective of this study, was to evaluate the use of CPM in the diet of laying hens in semi-intensive system.

2. Materials and Methods

Two experiments were carried out according to research ethics protocols from Resolution No. 879/08 of the National Council for Control and Animal Experimentation (CONCEA), with Protocol number 592/19 approved by the Animal Experimentation Committee of the Federal University of Piauí (CEEA - UFPI).

2.1. Obtention and production of the cactus pear meal

The cladodes of cactus pear *Miúda* (*Nopalea cochinilifera* (L.) Salm Dyck) and *Gigante* (*Opuntia ficus-indica* (L.) Mill) were harvested at the agrostology experimental station of the Federal University of Piauí (UFPI), Bom Jesus, Brazil. Two-year-old cladodes were harvested and transported to the Animal Nutrition Laboratory (LANA) of UFPI, where they were cut (5 to 3 cm thick) and distributed in trays in several layers. Pre-drying was performed in oven of forced ventilation at 55 °C for 72 h. The pre-dried material was weighed on an analytical scale and grounded in a Thomas Wiley SP-32 SLAPOR® mill in 1-mm mesh sieve and then it was stored.

2.2. Experiment I: Digestibility trial of the cactus pear meal varieties

2.2.1. Location and experimental conditions

The first experiment was carried out in the experimental poultry aviary of the Technical College of Bom Jesus (CTBJ) of the Federal University of Piauí (UFPI), at the Professora Cinobelina Elvas campus, in August 2019. The town is located at the following geographic coordinates: latitude 9°4' 27" South, longitude 44°21' 30" West and 277m altitude. The climate of the region is classified as "Aw" according to the Köppen (1928) classification [15]. The aviary had the sides closed by screens and was provided with mechanized external curtains and fans. The space for metabolic trials had four batteries of nine cages each, measuring 1.16 m x 1.10 m x 0.50 m of with screened floor, feeder and trough-type drinker and a removable bottom tray for collecting excreta.

2.2.2. Birds and experimental period

Seventy-two male and female Isa Label broiler chicks were used, and the treatments were distributed in a completely randomized design, with three diets and six replications of four birds each. The experimental period ranged from 24 to 32 days of age, with four days of adaptation to the diets and four days of total collection of excreta. The total excreta collection method was used, according to the procedures described by [16]. Before the beginning of the adaptation period, from 1 to 23 days of age, the birds were kept in separate batteries, and fed with a diet to meet the nutritional needs established by [17].

2.2.3. Diets and analyses

The treatments consisted of a reference diet (RD) based on corn and soybean meal (Table 1) and two test diets containing two varieties of cactus pear - Test Diet 1: 80% RD + 20% *Miúda* cactus pear meal (CPM) and Test Diet 2: 80% RD + 20% *Gigante* cactus pear meal.

Table 1. Composition of the reference diet from the metabolizable energy trial and digestibility coefficient of dry matter (DM), crude protein (CP) and mineral matter for male and female broiler chicks of the Isa Label strain from 24 to 32 days of age. Experiment I.

| Ingredients | % on as fed basis |
|--|-------------------|
| Corn grain (8.8% CP) | 61.8903 |
| Soybean meal (45%) | 32.4421 |
| Limestone | 0.4799 |
| Dicalcium Phosphate | 1.0465 |
| Common Salt | 0.1318 |
| Initial Bird Fit Core ¹ | 4.0000 |
| DL-Methionine | 0.0094 |
| Total | 100.0000 |
| Calculated Composition | |
| Calcium (%) | 1.1600 |
| Clorine (%) | 0.1505 |
| Bird Metabolizable Energy (kcal/kg) | 2.8769 |
| Available Phosphorus (%) | 0.4400 |
| Bird Digestible Lysin (%) | 0.9540 |
| Bird Digestible Methionine + Cystine (%) | 0.7000 |
| Crude Protein (%) | 20.0508 |
| Sodium (%) | 0.2410 |

¹ Guarantee levels per kg of product: Calcium (min): 100g/kg, Calcium (max): 200g/kg, Phosphorus (min): 40g/kg, Methionine (min): 32.276g/kg, Sodium (min): 44g/kg, Iron (min): 600mg/kg, Copper (min): 1,600mg/kg, Manganese (min): 1,440mg/kg, Zinc (min): 1,248mg/kg, Iodine (min): 28.8mg/kg, Selenium (min): 6.6mg/kg, Vitamin A (min): 140,000IU/kg, Vitamin D3 (min): 50,000IU/kg, Vitamin E (min): 260IU/kg, Vitamin K3 (min):

20mg/kg, Vitamin B1 (min): 12mg/kg, Vitamin B2 (min): 110mg/kg, Vitamin B5 (min): 32mg/kg, Vitamin B12 (min): 240mgc/kg, Niacin (min): 650mg/kg, Calcium Pantothenate (min):150mg/kg, Folic Acid (min):440mg/kg, Biotin (min): 0.6mg/kg, Choline Chloride (min): 4,563mg/kg, Phytase (min): 10,000FTIU/kg, Nicarbazin (min): 2,500mg/kg, Halquinol: 600mg/kg.

It was added 1% iron oxide to all diets to serve as a marker for the beginning and end of the collection period. Collections happened twice daily in trays covered with plastic material. After collection, the excreta were weighed, packed, identified, and frozen at 18 °C until the end of the collection period. Subsequently, the excreta were thawed, weighed, homogenized, and a sample was removed, weighed, and dried in forced ventilation oven at 65 °C for 72 h. The oven-dried samples were weighed, grounded and put in containers for laboratory analysis.

The contents of dry matter (DM), mineral matter (MM), crude protein (CP) and gross energy (GE) were determined in all samples following the methods of [18]. With the laboratory results, the apparent metabolizable energy (AME), dry matter digestibility coefficient (DMDC), crude protein digestibility coefficient (CPDC) and mineral matter digestibility coefficient (MMDC) were determined according to [16].

The results obtained from the analyses for ingested and excreted diet were used to determine the apparent metabolizable energy corrected for nitrogen balance (AMEn) of the experimental diets [19]. The same principle was used to calculate the DMDC, CPDC and MMDC [19].

$$\text{Nutrient digestibility coefficient (NDC)}_{RefB} = \frac{(\text{Nutrient ingested} - \text{Nutrient excreted}) * 100}{\text{Nutriente ingested}}$$

$$\text{NDC (Cactus Pear - CP)} = \text{NDC (Ref)} + \frac{(\text{NDC(diet with CP)} - \text{NDC(Ref)})}{\% \text{ CP substitution}}$$

2.3. Experiment II: production performance and egg quality

2.3.1. Location and experimental conditions

The second experiment was carried out at the School Farm Alvorada do Gurguéia (FEAG) of the Federal University of Piauí, located in the town Alvorada do Gurguéia, Piauí, Brazil, from August to October 2020. The town is in the Upper Middle Gurguéia microregion at the following geographic coordinates: latitude 8°22'34" South, longitude 43°51'23" West and 220 m of altitude. The climate of the region is classified as "Aw" according to the Köppen (1928) classification [15]

The laying hens were housed in a brick aviary with clay tile roof, in 20 boxes measuring 2 m x 1 m, and each box had access to an external paddock of 9.80 m x 5.45 m surrounded by wire. The boxes were equipped with side curtains with manual adjustment system, bedding of rice straw, a nest of 30 cm x 35 cm covered with rice straw, a tubular feeder and a pendular drinking trough.

2.3.2. Birds and experimental period

Initially, 80 female chicks of the Bankiva GLK strain of one day of age were housed in prepared boxes, and fed a diet based on corn and soybean meal according to the guidelines of the Embrapa Brown Egg Colonial Laying Hen Management Guide [20] by life stage: brood (1st to 6th week); rearing (7th to 15th week), and pre-laying (16th to 31st week). During the pre-laying phase, egg production was monitored until approximately 80% laying was obtained to begin the experimental phase. At 31 weeks of age, the hens were weighed for plot assembly, according to the experimental design, and housed for adaptation to new groups. At 32 weeks of age, hens were reweighed, presenting an average initial weight of 1.647 kg and distributed in 20 boxes of similar weights. At the end of the experimental period, at 40 weeks of age, the hens were weighed again, presenting an average final weight of 1.679 kg. The treatments were distributed in a completely randomized design with four treatments, and five replications of four hens each. The study lasted for 63 days, divided into three periods of 21 days each

2.3.3. Diets and Management

The experimental diets (Table 2) were formulated according to the nutritional requirements suggested by management recommendation guide Free-range laying hens (32th to 40th week) of brown eggs [20]. Nutritional values of ingredients according to [21], except for the cactus pear, which considered the composition of calcium, phosphorus, sodium, potassium, NDF and ADF determined by [6], and the total amino acid composition determined by [22], and digestible amino acids by the digestibility coefficient of foods with fiber content similar to cactus pear of [21] (Table 3). The cactus pear used in this experiment was the *Miúda* (*Nopalea cochinilifera* (L.) Salm Dyck), from a macro project that prioritizes savings in water use through plant fertilization using hydrogels [23,24]. The composition and metabolizable energy of cactus pear (Miúda) analyzed in the first experiment were considering for formulate experimental diets, for maintained same nutrient levels in all treatments.

The diets consisted of levels of CPM: Treatment 1: Control diet based on corn and soybean meal; Treatment 2, 3 and 4: Diet with 3, 6 and 9% of CPM, respectively.

Table 2. Composition of experimental diets with levels of *Miúda* cactus pear meal (CPM) for the feeding of laying hens from the 32nd to the 40th week of age in semi-intensive system: Experiment II.

| Ingredients | CPM Levels (%) | | | |
|--|----------------|---------|---------|---------|
| | 0 | 3 | 6 | 9 |
| Corn grain | 65.650 | 62.650 | 59.650 | 56.650 |
| Soybean meal | 16.264 | 16.914 | 17.564 | 18.214 |
| Limestone | 8.747 | 8.498 | 8.249 | 7.999 |
| Wheat bran | 5.533 | 4.372 | 3.212 | 2.052 |
| Dicalcium Phosphate | 2.141 | 2.165 | 2.189 | 2.213 |
| Soybean oil | 0.546 | 1.288 | 2.030 | 2.772 |
| Common salt | 0.375 | 0.376 | 0.377 | 0.377 |
| Vitini-bird* | 0.300 | 0.300 | 0.300 | 0.300 |
| DL-methionine | 0.214 | 0.218 | 0.223 | 0.228 |
| L-lysine HCl | 0.192 | 0.180 | 0.168 | 0.156 |
| L-threonine | 0.025 | 0.026 | 0.026 | 0.027 |
| L-tryptophan | 0.013 | 0.012 | 0.011 | 0.010 |
| CPM | 0.000 | 3.000 | 6.000 | 9.000 |
| Total | 100.000 | 100.000 | 100.000 | 100.000 |
| Energetic and nutritional composition | | | | |
| Crude protein (%) | 14.15 | 14.15 | 14.14 | 14.14 |
| Bird. Met. Energy (kcal/kg) | 2750 | 2750 | 2750 | 2750 |
| Calcium (%) | 3.900 | 3.900 | 3.900 | 3.900 |
| Total Phosphorus (%) | 0.702 | 0.692 | 0.682 | 0.672 |
| Available Phosphorus (%) | 0.500 | 0.500 | 0.500 | 0.500 |
| Sodium (%) | 0.160 | 0.160 | 0.1600 | 0.160 |
| Clorine (%) | 0.295 | 0.292 | 0.289 | 0.286 |
| Potassium (%) | 0.614 | 0.682 | 0.750 | 0.812 |
| Bird Dig. Methionine (%) | 0.414 | 0.416 | 0.419 | 0.422 |
| Bird Dig. Met. + Cyst. (%) | 0.620 | 0.620 | 0.620 | 0.620 |
| Bird Dig. Lysin (%) | 0.750 | 0.750 | 0.750 | 0.750 |
| Bird Dig. Threonine (%) | 0.500 | 0.500 | 0.5000 | 0.500 |
| Bird Dig. Tryptophane (%) | 0.160 | 0.160 | 0.1600 | 0.160 |
| Linoleic Acid (%) | 1.750 | 2.070 | 2.391 | 2.711 |

* Guarantee levels per kg of product: Methionine (min): 160g/kg, Iron (min): 5,760mg/kg, Copper (min): 1,600mg/kg, Manganese (min): 11.52g/kg, Zinc (min): 12g/kg, Iodine (min): 288mg/kg, Selenium (min): 60mg/kg, Vitamin A (min): 2,000,000IU/kg, Vitamin D3 (min): 600,000IU/kg, Vitamin E (min): 5,400IU/kg, Vitamin K3 (min): 300mg/kg, Vitamin B1 (min): 300mg/kg, Vitamin B2 (min): 1,400mg/kg, Vitamin B6 (min):

600mg/kg, Vitamin B12 (min): 4,000mcg/kg, Niacin (min): 6,400mg/kg, Calcium Pantothenate (min): 2,600mg/kg, Folic Acid (min): 400mg/kg, Biotin (min): 20mg/kg, Choline Chloride (min): 66g/kg, Halquinol: 6,000mg/kg.

Table 3. Chemical composition of CPM. Experiment II.

| Component | Composition* | Component | Composition |
|-------------------------|--------------------------------|----------------------|---------------------------------|
| Dry matter (DM) | 124.6 g kg ⁻¹ | Dig. Methionine | 0.25 g kg ⁻¹ of DM** |
| Crude protein | 33.7 g kg ⁻¹ of DM | Dig. Lysine | 1.19 g kg ⁻¹ of DM** |
| Ether extract | 14.0 g kg ⁻¹ of DM | Dig. Threonine | 0.52 g kg ⁻¹ of DM** |
| Mineral matter | 151.3 g kg ⁻¹ of DM | Dig. Tryptophane | 0.18 g kg ⁻¹ of DM** |
| Organic matter | 871.2 g kg ⁻¹ of DM | Sodium | 0.05 g kg ⁻¹ of DM* |
| Total carbohydrates | 822.1 g kg ⁻¹ of DM | Calcium | 29.3 g kg ⁻¹ of DM* |
| Neutral detergent fiber | 224.6 g kg ⁻¹ of DM | Total Phosphorus | 0.78 g kg ⁻¹ of DM* |
| Acid detergent fiber | 189.7 g kg ⁻¹ of DM | Available Phosphorus | 0.78 g kg ⁻¹ of DM |

*by [6], **determined by [22] used digestibility coefficient of feeds with fiber content similar to cactus pear of [21].

Feed and water were offered at will during the experimental period. Egg collection was performed manually at 1pm and 5pm. After egg collection at 5 pm, the nests were closed and reopened in the morning to avoid dirt. Lighting was established according to the age of the hens and the sunrise and sunset times of the region, using a photoperiod of 14 h of light and 10 h of dark, with 12 h of natural light and 2 h of artificial light. The light supply was operated manually, with half being offered in the morning (5 to 6 am) and the other half in the evening (6 to 7 pm). Air temperature and humidity were monitored by two digital maximum and minimum thermo-hygrometers (TOMATE® PD003) located inside two boxes on opposite sides at the height of the hens' backs. Climatic data were collected at 6 am throughout the experimental period.

2.3.4. Productive performance

The following performance parameters were evaluated: feed intake (FI) (g/bird/day), water intake (WI) (mL/bird/day), egg production (EP) (%), egg weight (EW) (g), egg mass (EM) (g), conversion per egg mass (CEM)(kg/kg), and conversion per egg dozen (CED) (kg/dozen). The variables were analyzed in three cycles of 21 days each. The analyses occurred at the 34th, 37th, and 40th week (17 days after the beginning experimental feed supply). Feed intake was calculated by the difference between the amount of feed provided and the experimental leftovers, weighed at the beginning and end of each 21-day period. Water intake was calculated by measuring the supplied and leftover water for four consecutive days of each period. Egg production was recorded during the three 21-day periods on laying sheets twice daily and obtained by dividing the sum of eggs per period and per plot by the number of birds and multiplied by 100. On the last four days of each period, eggs from each plot were weighed individually to obtain the average egg weight. Egg mass was calculated as the product of egg production and average egg weight per plot. Feed conversion per egg mass was calculated by the ratio between feed intake and egg mass produced, and the conversion per egg dozen was calculated by the ratio between feed intake and production in dozens.

2.3.5. Egg quality

Variables were analyzed in three cycles of 21 days each. The analyses occurred in the 34th, 37th and 40th week (17 days after the beginning of the experimental feed supply). Quality variables were evaluated in three eggs per plot, from 18 to 21 days of each period, obtaining the parameters: yolk diameter (YD) (mm) which was determined by a Pantec® digital pachymeter model 150MM/6; Yolk Percentage (YOLK), Albumen Percentage (ALB), Shell Percentage (SHELL) which were obtained by dividing the component weight by the egg weight, and multiplying the result by 100; shell thickness (ST) (mm) which was measured in three parts (apical, equatorial and basal) using a digital pachymeter; specific weight (SW) (g/cm³) which was performed by the salt flotation method as described by [25], where the eggs were immersed in salt solutions with densities ranging from 1.070

to 1.090 with an interval of 0.0025, with densities adjusted using petroleum densimeter; and Haugh Unit (HU) which was determined using the equation: $HU = 100 \log (H + 7.57 - 1.7W0.37)$, where, H = albumen height (mm); and W = egg weight (g) [26,27].

On the last collection day of each period, two eggs per plot were analyzed for Shell Resistance (SR), and Yolk Resistance (YR) (kgf) through the Texture Analyzer Brookfield® (model CT3 50 kg) connected to a computer equipped with TextureLoader® software. Three color parameters were evaluated: L*, a* and b*. The a* value characterizes coloration in the red (+a*) to green (-a*) region, the b* value indicates coloration in the yellow (+b*) to blue (-b*) range. And, the L* value gives the brightness, ranging from white (L=100) to black (L=0) [28]. The coloration parameters were measured at three different points on the egg yolk with the aid of a Minolta CR-400® portable colorimeter in the CIELab system.

2.3.6. Analysis of egg texture profile and color of the cooked yolk

Ten eggs from each treatment were used for egg texture profile analysis, in which the variables analyzed were hardness, cohesiveness, elasticity, gumminess, whole egg chewiness, and yolk color parameters. Eggs were cooked by treatment for 10 min and placed in cold water for 3 min to be peeled. Whole egg texture was done using the Texture Analyzer Brookfield® (model CT3 50 kg) connected to a computer equipped with TextureLoader® software. The speed of the slide test was 2.00 mm/s in 2 cycles. After that, the yolk was separated manually to be analyzed for coloration by a Minolta CR-400® portable colorimeter, in the CIELab system.

2.3.7. Egg composition, fatty acids and cholesterol in the yolk

Cooked yolk and albumen samples were frozen after texture profile analysis. Subsequently, they were thawed, weighed, prepared to be freeze-dried in for 72 h, and then ground in a small domestic mixer to a powder. The freeze-dried powdered samples were analyzed for DM, CP, and MM content through the methods of [18].

The fatty acids (FAs) in the samples were methylated according to [29]. The resulting fatty acid methyl ester was determined using a gas chromatograph (model Focus GC; Thermo Scientific, Milan, Italy), equipped with a flame ionization detector and SP 2560 fused silica capillary column (100 m x 25 mm x 0.2 µm film thickness; Supelco, Bellefonte, Pennsylvania). Hydrogen was used as carrier gas (1 mL/min) and nitrogen as an auxiliary gas. The detector and injector temperatures were set at 250 °C, with a split ratio of 15:1. The oven temperature was set to 70 °C for 4 min, increased by 13 °C/min until 175 °C, held for 27 min, then increased by 4 °C/min until 215 °C and held for 31 min. FAMEs were identified by comparing three FAME references (Supelco FAME mix C4-C24, CLA trans-9, cis 11 16413, and CLA trans-10, cis 12 04397; Sigma Aldrich).

Quantification of total cholesterol (TC) in yolk from the 32nd to the 40th week of age was performed according to the methodology described by [30]. Saponification of 0.5 mL of lipid extract was performed in a 50-mL Falcon tube, adding 10 mL of potassium hydroxide solution (KOH 2%) in 90% ethanol. Then the tubes were placed in water bath at 80 °C under stirring for 15 min. Subsequently, 5 mL of distilled water was added and allowed to cool. For extraction of unsaponifiable matter, 10 mL of hexane was added stirring in vortex for 1 min. After separation, the entire hexane phase was transferred to another Falcon tube and the extraction was repeated two more times. About 4 mL of hexanoic extract was collected and evaporated in a water bath at 55 °C, added 6 mL of saturated acetic acid in iron sulfate, cooled in a gel bath, and vortexed for 1 min. Immediately after, about 2 mL of sulfuric acid was added and cooled to 26 °C. After 10 min the reading was taken in a spectrophotometer at 490nm. Different concentrations from 0 to 200ppm of purified cholesterol (sigma) were used in the standard cholesterol curve and the absorbances were performed in a UV-VIS® spectrophotometer.

2.3.7. Economic Viability

The economic viability analysis was performed through the price of feed per kilogram (kg, R\$), feeding cost per dozen eggs (FCDZ, R\$/each) and relative gross margin (RGM, %), that is, the gross margin (GM, R\$) of the treatments using CPM in comparison to the GB of the control treatment. The determination of RGM was performed according to [31], considering only the variable costs of feeding, since the fixed costs were the same for all treatments.

The input prices (R\$/kg) used for the price calculations of the kilogram of feed were: corn = R\$ 1.10; soybean meal = R\$ 3.70; wheat bran = R\$ 1.30; soybean oil = R\$ 5.56; dicalcium phosphate = R\$ 5.40; limestone = R\$ 4.00, common salt = R\$ 0.80; vitamin supplement = R\$ 6.40; DL-methionine = R\$ 15.00; L-lysine HCL = R\$ 7.00; L-threonine = R\$ 8.00; L-tryptophan = R\$ 60.00, L-arginine = R\$ 95.00 and *Miúda* cactus pear meal = R\$ 0.20. To calculate the price of CPM it was taken into consideration only the expenses of handling the product (labor). The price of feed per kilogram (R\$/kg) was obtained by multiplying the price by the quantity of ingredients used per treatment/100; feeding cost per dozen eggs (FCDZ, R\$/ dozen) was obtained by the price of feed kilogram and bird intake divided by 12. The price per dozen eggs (PDZ, R\$/per dozen) was obtained by R\$ 8.00 in the local market of Bom Jesus-PI at the end of the experiment (October 2020).

2.4. Statistical Analyses

Data of the variables obtained were subjected to analysis of variance and compared by SNK test at 5% probability. The estimates of the use of cactus pear were established by polynomial regression for the significant variables. The SAS® software university edition was used for the analyses.

3. Results

3.1. Metabolizable Energy and Digestibility Coefficient of Cactus Pear Meal

The contents of 3,549 and 3,399 kcal/kg GE and 1,402 and 1,142 kcal/kg AME (Figure 1) were found for the varieties of *Miúda* cactus pear meal (CPM) and *Gigante* cactus pear meal, respectively for chicks aged 24 to 32 days. The DMDC value of CPM (43.5%) was higher than that of *Gigante* (27.9%); CPDC of CPM (36.0%) was also higher than that of *Gigante* (25.8%) which was again observed for MMDC of CPM (34.5%) in comparison to *Gigante* (19.2%) (Figure 2). Comparing DMDC, CPDC and MMDC, it can be observed that the variety *Miúda* showed higher nutritional digestibility than *Gigante*.

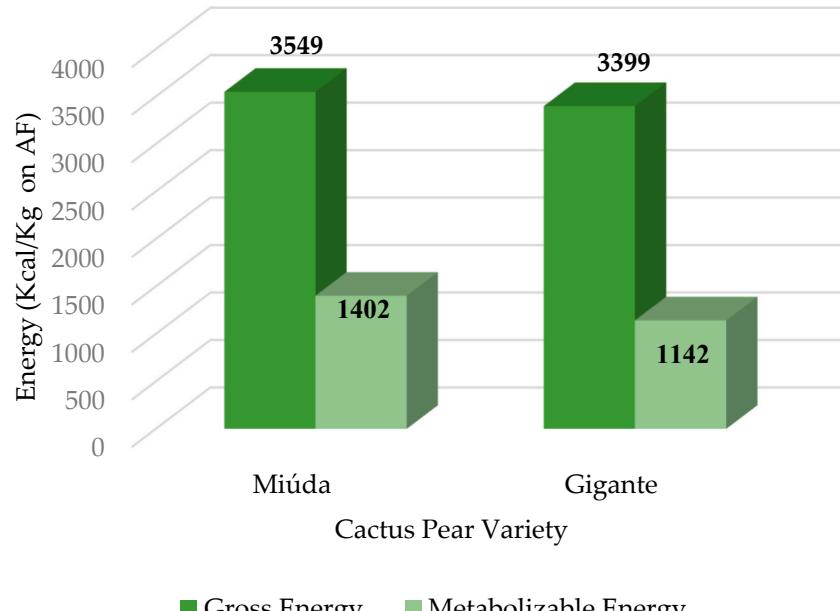


Figure 1. Gross energy and metabolizable energy of the meal of cactus pear varieties *Miúda* and *Gigante* for male and female broiler chicks of the Isa Label strain from 24 to 32 days of age. Experiment I.

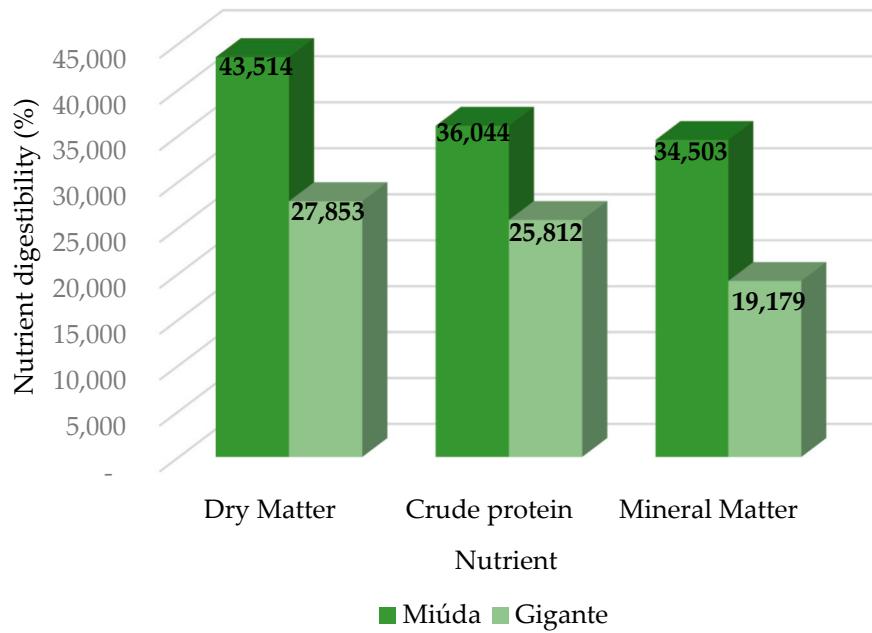


Figure 2. Digestibility coefficient of dry matter, crude protein and mineral matter of the meal of cactus pear varieties *Miúda* and *Gigante* for male and female broiler chicks of the Isa Label strain from 24 to 32 days of age. Experiment I.

3.2. Climate Data

Temperature and air relative humidity averages for the entire experimental phase of the second experiment are presented in Table 4. The relationship between temperature and nutrition should be analyzed and taken into consideration in the rearing of commercial laying hens, since the variation in ambient temperature regulates mainly feed intake. Temperature did not affect the production and egg quality data.

Table 4. Indoor temperature and humidity data from the 32nd to the 40th week of age of laying hens in semi-intensive system. Experiment II.

| Maximum Temperature C° | Minimum Temperature C° | Maximum Humidity % | Minimum Humidity % |
|------------------------|------------------------|--------------------|--------------------|
| 39.6 | 21.9 | 64.0 | 13.4 |

3.3. Productive Performance

The diets containing levels of CPM did not promote significant effects on the variables egg production, feed intake, water intake, egg weight, egg mass, conversion per egg mass and conversion per egg dozens of laying hens in semi-intensive system from the 32nd to the 40th week of age (Table 5).

Table 5. Effect of the use of levels of CPM on Egg Production (EP), Feed Intake (FI), Water Intake (WI), Egg Weight (EW), Egg Mass (EM), Conversion per Egg Mass (CEM) and Conversion per Egg Dozen (CED). Experiment II.

| Variables | CPM Levels (%) | | | | ANOVA | P reg | CV |
|------------------|----------------|--------|--------|--------|--------|-------|------|
| | 0 | 3 | 6 | 9 | | | |
| EP (%) | 89.68 | 93.95 | 95.15 | 93.73 | 0.3134 | ns | 4.60 |
| FI (g/bird/day) | 114.53 | 109.82 | 115.97 | 109.25 | 0.6058 | ns | 8.03 |
| WI (mL/bird/day) | 319.41 | 356.15 | 348.67 | 351.03 | 0.4295 | ns | 9.67 |
| EW (g) | 55.63 | 53.82 | 54.19 | 53.45 | 0.4142 | ns | 3.47 |
| EM (g) | 49.91 | 50.55 | 51.61 | 50.10 | 0.8791 | ns | 6.65 |
| CEM (g/g) | 2.31 | 2.17 | 2.26 | 2.18 | 0.7026 | ns | 8.36 |
| CED (kg/dozen) | 1.54 | 1.40 | 1.47 | 1.40 | 0.2898 | ns | 8.03 |

CV = coefficient of variation, ns = not significant.

3.4. Egg Quality

The effects of CPM levels on egg quality parameters of laying hens reared in semi-intensive system are presented in Table 6. There was a linear reduction between treatments in yolk diameter (YD = 34.14 - 0.0991CPM, $R^2 = 0.9864$) that is, yolk diameter reduced linearly as the level of CPM increased in the diet. Shell resistance (SR= 4363.94 - 50.9031CPM, $R^2 = 0.9178$), yellow coloration ($b^* = 58.35 - 0.8404$ CPM, $R^2 = 0.8853$) and red coloration ($a^* = 9.68 - 0.3897$ CPM, $R^2 = 0.9707$) also decreased linearly as the level of CPM increased. On the other hand, there was a linear increase in albumen percentage (ALB= 62.00 + 0.0881CPM, $R^2 = 0.90$) as the levels of CPM increased in the diet. However, there was no significant effect on yolk percentage, shell percentage, shell thickness, specific weight, Haugh Unit, yolk strength and yolk brightness with increasing levels of CPM.

Table 6. Effect of the use of levels of CPM on Yolk Diameter (YD), Yolk Percentage (YOLK), Albumen Percentage (ALB), Shell Percentage (SHELL), Shell Thickness (ST), Specific Weight (SW), Haugh Unit (HU), Shell Resistance (SR), Yolk Resistance (YR) and Yolk pigmentation parameters L*, a* and b*. Experiment II.

| Variables | CPM Levels (%) | | | | ANOVA | P reg | CV |
|-------------------------|----------------|---------|---------|---------|--------|----------------------|-------|
| | 0 | 3 | 6 | 9 | | | |
| YD (mm) | 34.27 | 33.69 | 33.50 | 33.33 | 0.0887 | 0.0127 ^L | 1.50 |
| YOLK (%) | 24.11 | 23.63 | 24.10 | 23.42 | 0.1948 | ns | 2.43 |
| ALB (%) | 62.07 | 62.11 | 62.61 | 62.78 | 0.2431 | 0.0447 ^L | 0.92 |
| SHELL (%) | 10.29 | 10.41 | 10.36 | 10.31 | 0.8984 | ns | 2.22 |
| ST (mm) | 0.38 | 0.38 | 0.38 | 0.38 | 0.9599 | ns | 3.53 |
| SW (g/cm ³) | 1.0922 | 1.0932 | 1.0924 | 1.0927 | 0.9113 | ns | 0.19 |
| HU | 90.62 | 93.39 | 91.54 | 93.10 | 0.3660 | ns | 2.78 |
| SR (g/cm ²) | 4311.81 | 4254.08 | 4115.06 | 3856.69 | 0.2370 | 0.0425 ^L | 7.94 |
| YR (g/cm ²) | 17.33 | 15.65 | 15.44 | 14.93 | 0.7362 | ns | 17.23 |
| L* | 59.49 | 59.82 | 60.58 | 60.37 | 0.7348 | ns | 2.53 |
| a* | 9.67 | 8.75 | 6.99 | 6.34 | 0.0004 | <0.0001 ^L | 11.73 |
| b* | 59.11 | 52.50 | 52.53 | 51.78 | 0.0266 | 0.0049 ^L | 6.71 |

L = linear, ns = not significant, CV = coefficient of variation.

3.5. Analysis of egg texture profile and color of the cooked yolk

The effects of CPM levels on the parameters of egg texture profile and yolk color of cooked eggs are presented in Table 7. There was linear increase cohesiveness (COHE= 0.384 + 0.0085CPM, $R^2=0.16$), which means that as the level of CPM increased the cohesiveness of the cooked egg also increased. The red coloration of the yolk reduced linearly ($a = 6.405 - 0.305$ CPM, $R^2=0.29$) as the level of CPM

increased. While for yellow coloration of the yolk there was a quadratic effect ($b = 41.965 - 2.917\text{CPM} + 0.223\text{CPM}^2$, $R^2=0.54$), with the color b reducing up to 6.52% with the use of CPM.

Table 7. Effect of the use of levels of CPM on Conversion Indicator (CI), Hardness (HARD), Cohesiveness (COHE), Elasticity (ELAS), Gumminess (GUMM), Chewiness (CHEW) and yolk pigmentation parameters L*, a* and b*. Experiment II.

| Variables | CPM Levels (%) | | | | ANOVA | P reg | CV |
|-----------|----------------|-------|-------|-------|--------|---------------------|-------|
| | 0 | 3 | 6 | 9 | | | |
| CI | 0.995 | 0.998 | 0.996 | 0.988 | 0.7857 | ns | 2.26 |
| HARD | 189.5 | 195.8 | 186.7 | 191.0 | 0.7857 | ns | 17.36 |
| COHE | 0.398 | 0.380 | 0.452 | 0.458 | 0.0299 | 0.0122 ^L | 15.41 |
| ELAS | 6.0 | 6.0 | 6.3 | 6.5 | 0.7729 | ns | 17.31 |
| GUMM | 80.8 | 77.8 | 86.0 | 86.1 | 0.7127 | ns | 20.85 |
| CHEW | 6.1 | 5.9 | 6.3 | 6.2 | 0.9813 | ns | 41.39 |
| Col L | 81.9 | 82.1 | 84.2 | 84.1 | 0.4005 | ns | 4.75 |
| Col a | 6.60 | 5.30 | 4.26 | 3.91 | 0.0054 | 0.0006 ^L | 33.88 |
| Col b | 42.1 | 34.5 | 33.1 | 33.5 | 0.001 | 0.0017 ^Q | 9.98 |

L = linear, Q = quadratic, ns=not significant, CV=coefficient of variation.

3.6. Egg composition, fatty acids and cholesterol in the yolk

The effects of levels of CPM on egg chemical composition, fatty acids and total cholesterol in the yolk are presented in Table 8. It was found decreasing effect on albumen MM ($MM_{\text{Albumen}} = 2.699 - 0.1591\text{CPM} + 0.0134\text{CPM}^2$, $R^2=0.75$). For albumen DM and CP there were no significant effects of CPM levels. There were also no significant effects for yolk DM, MM and CP of the increasing levels of CPM for laying hens in semi-intensive system. The use of CPM levels did not promote significant effect on Total yolk FAs, but linearly reduced Myristic = $0.4758 - 0.01002\text{CPM}$, $R^2=0.5$, Myristoleic = $0.0802 - 0.00126\text{CPM}$, $R^2=0.68$ and Oleic = $39.5211 - 0.1828\text{CPM}$, $R^2=0.61$. There was also a reduction in Palmitic = $27.423 - 0.5864\text{CPM} + 0.0386\text{CPM}^2$, $R^2=0.84$, Palmitoleic = $4.2453 - 0.4056\text{CPM} + 0.0274\text{CPM}^2$, $R^2=0.98$ and Vaccenic = $2.223 - 0.0939\text{CPM} + 0.00462\text{CPM}^2$, $R^2=0.98$. Cactus pear levels promoted linear increase in α -linolenic = $0.4163 + 0.0253\text{CPM}$, $R^2=0.84$ and other FAs = $4.598 + 0.0573\text{CPM}$, $R^2=0.59$, as well as increase in Stearic = $7.575 + 0.2237\text{CPM} - 0.0196\text{CPM}^2$, $R^2=0.68$, Linoleic = $12.368 + 0.8296\text{CPM} - 0.03731\text{CPM}^2$, $R^2=0.99$ and Arachidonic = $1.7061 + 0.0523\text{CPM} - 0.0046\text{CPM}^2$, $R^2=0.86$. The total Cholesterol in the yolk of laying hens fed CPM in semi-intensive system also increased linearly ($TC = 397.4 + 20.37\text{CPM}$, $R^2=0.77$).

Table 8. Effect of the use of levels of CPM on dry matter (DM), mineral matter (MM), and crude protein (CP) in albumen and yolk, and profile of fatty acids (FAs) and total cholesterol (TC) of yolk of eggs from laying hens in semi-intensive system. Experiment II.

| CPM Levels (%) | 0 | 3 | 6 | 9 | ANOVA | P reg | CV |
|-----------------|---------|-------|-------|-------|---------|---------------------|-------|
| Variables | Albumen | | | | | | |
| DM (%) | 20.92 | 17.47 | 23.16 | 19.95 | 0.0061 | ns | 6.68 |
| MM (%) | 2.68 | 2.41 | 2.12 | 2.38 | 0.0094 | 0.0202 ^Q | 4.58 |
| CP (%) | 9.45 | 9.22 | 9.31 | 9.41 | 0.8168 | ns | 3.35 |
| Yolk | | | | | | | |
| DM (%) | 49.9 | 49.74 | 49.94 | 50.51 | 0.6242 | ns | 2.07 |
| MM (%) | 3.12 | 3.16 | 3.16 | 3.20 | 0.9976 | ns | 14.59 |
| CP (%) | 3.34 | 3.75 | 3.29 | 3.33 | 0.3808 | ns | 10.01 |
| Total FAs (%) | 45.94 | 47.20 | 45.54 | 46.22 | 0.7633 | ns | 4.23 |
| Myristic (%) | 0.486 | 0.433 | 0.410 | 0.392 | 0.0678 | 0.0074 ^L | 8.61 |
| Myristoleic (%) | 0.079 | 0.080 | 0.069 | 0.070 | <0.0001 | 0.0010 ^L | 2.15 |
| Palmitic (%) | 27.51 | 25.76 | 25.54 | 25.18 | 0.0004 | 0.0202 ^Q | 1.51 |

| | | | | | | | |
|-------------------------|-------|-------|-------|-------|---------|----------------------|------|
| Palmitoleic (%) | 4.27 | 3.21 | 2.87 | 2.79 | <0.0001 | <0.0001 ^Q | 1.76 |
| Stearic (%) | 7.58 | 8.05 | 8.22 | 7.99 | 0.0201 | 0.0090 ^Q | 2.45 |
| Oleic (%) | 39.26 | 39.46 | 38.24 | 37.84 | 0.0092 | 0.0028 ^L | 1.25 |
| Vaccenic (%) | 2.22 | 1.97 | 1.83 | 1.75 | <0.0001 | 0.0006 ^Q | 1.49 |
| Linoleic (%) | 12.38 | 14.47 | 16.05 | 16.79 | <0.0001 | 0.0002 ^Q | 1.33 |
| α -linolenic (%) | 0.395 | 0.517 | 0.580 | 0.627 | 0.0004 | <0.0001 ^L | 7.16 |
| Arachidonic (%) | 1.701 | 1.837 | 1.836 | 1.806 | 0.0002 | 0.0003 ^Q | 1.22 |
| OTHER FAs (%) | 4.51 | 4.86 | 5.04 | 5.02 | 0.0113 | 0.0036 ^L | 3.27 |
| TC (mg/100g) | 388 | 469 | 534 | 572 | <0.0001 | <0.0001 ^L | 8.75 |

L = linear, Q = quadratic, ns=not significant, CV=coefficient of variation.

3.7. Economic Viability

Economic viability data are presented in Table 9. Feed intake was greater for birds receiving the treatment with 6% CPM while the treatment with 9% CPM had the lowest intake. The price for formulation of diet with 9% CPM was higher. Feeding cost, egg dozen and cost per egg dozen were higher for the treatment with 6% CPM when compared to the other treatments. The price per dozen eggs was R\$8.00 according to the region and season of the experiment. The gross income was higher when using the treatment with 6% CPM, while the gross margin was higher for the treatment with 3% CPM.

Table 9. Effect of the use of levels of CPM on the economic viability parameters of laying hens in semi-intensive system. Experiment II.

| Variables | CPM Levels % | | | |
|--------------------------------|--------------|--------|--------|--------|
| | 0 | 3 | 6 | 9 |
| Feed intake, kg/bird | 7.216 | 7.150 | 7.342 | 7.022 |
| Price/kg of feed, R\$/kg | 1.969 | 1.983 | 1.997 | 2.011 |
| Feeding cost, R\$/bird | 14.209 | 14.179 | 14.664 | 14.122 |
| Egg dozen, dz/bird | 4.708 | 4.953 | 5.003 | 4.943 |
| Feeding cost/Egg dozen, R\$/dz | 3.029 | 2.861 | 2.932 | 2.857 |
| Price of egg dozen, R\$ | 8.000 | 8.000 | 8.000 | 8.000 |
| Gross income, R\$ | 37.665 | 39.625 | 40.021 | 39.542 |
| Gross margin, R\$ | 23.458 | 25.446 | 25.358 | 25.420 |
| Relative gross margin, % | 100.00 | 114.97 | 109.49 | 109.54 |

4. Discussion

The contents of 3,549 and 3,399 kcal/kg of GE and 1,402 and 1,142 kcal/kg AME (Figure 1) were found in the present study for the varieties of CPM and *Gigante* cactus pear meal. In the study of [32], found values of 4,009, 3,757 and 3,945 kcal/kg GE; 3,144, 3,019 and 1,624 kcal/kg AME for corn, sorghum, and wheat bran, respectively for chicks aged 26 to 33 days. The authors [33], found new gross energy values in corn grain (3,884 kcal/kg), wheat grain (3,867 kcal/kg) and sorghum grain (3,987 kcal/kg). As for the metabolizable energy, values of 3,719 kcal/kg for corn, 3,265 kcal/kg for wheat, and 3,695 kcal/kg for sorghum were found for chicks aged 22 to 28 days. Whereas [34] found metabolizable energy values of 1,259 kcal/kg and 1,316 kcal/kg for corn and wheat, respectively, for 7-, 14-, 21-, 28-, and 35-day-old birds. However, these differences in gross energy and metabolizable energy of those feedstuffs were expected, as there are variations in soil conditions, climate, raw material obtention, storage time, processing, age of the birds, physiological state, methodology used, and chemical composition [33,35,36].

The AME value of CPM (1,402 kcal/kg) was higher than that of *Gigante* cactus pear meal (1,142 kcal/kg). Such differences may be related to the species and chemical composition of the varieties of cactus pear that may interfere in the metabolizable energy. The AME value of cactus pear *Miúda* stood

out due to its bromatological composition that presented lower contents of soluble fiber when compared to *Gigante* [7,37,38].

The authors [39] states that the metabolizable energy is directly and positively affected by the composition of the feed in starch, fat and protein and negatively affected by the structural carbohydrates of the plant. The AME value of *Gigante* cactus pear meal may have been negatively affected by the soluble fiber content (NSPs and pectin) mainly the high content of pectin [38] and its high water solubility [40]. These physicochemical characteristics of the soluble fiber fraction result in increasing viscosity of the digest. High viscosity decreases the diffusion rate of endogenous enzymes in the digest, which will reduce nutrient digestion. In addition, the highly viscous digesta will have less interaction with enzymes in the brush border membrane, which also decreases digestibility and nutrient utilization [40,41].

On the other hand, the AME value of CPM was positively affected by the high contents of non-fiber carbohydrates [7,38,42,43] mainly starch [7], being the main source of energy for birds. These results are attributed to the higher intake of non-fiber carbohydrates, which consequently provided higher energy intake [44].

CMDC, CPDC and MMDC of variety CPM were higher when compared to *Gigante* cactus pear meal (Figure 2), and this is presumably due to the nutritive value that varies between cultivars [45]. The DMDC value of CPM (43.5%) was higher than *Gigante*'s cactus pear meal (27.9%). The reduced dry matter digestibility of the cactus pear varieties, mainly for the *Gigante* cactus pear meal may be attributed to considerable amounts of NSP that cannot be digested by birds because they lack endogenous enzymes. Soluble NSPs can increase the viscosity of the digestate and reduce nutrient digestibility [46]. The superiority in DMDC of CPM can be attributed to the sugar and starch contents [7,38], since the concentration of these carbohydrates contributes considerably to high palatability, which explains the higher dry matter digestibility of that variety, corroborating the results represented in Figure 2.

The CPDC values of CPM (36.0%) and *Gigante* cactus pear meal (25.8%) found in this study were lower than the values found by [47], who found protein digestibility coefficient 92.69% for corn and 91.41% for sorghum for Isa Label chickens, from 28 to 35 days. In other research, [48] found protein digestibility coefficient of 75.24% for corn and 87.84% for sorghum for Label Rouge birds. These significant differences in the CPDC of cactus pear meal, corn and sorghum are related to the chemical composition of the feed (antinutritional factors and the amount of fiber), in addition to the strain of the birds that can influence the digestibility of nutrients. The lower CPDC may be because the cactus pears are from the genera *Opuntia* (*Gigante*) and *Nopalea* (*Miúda*), that is, the genus influences composition and the composition influence nutrient utilization. Cactus pear variety *Gigante* presents a higher concentration of soluble fibers [7,37,38]. Soluble fibers impair protein digestibility because they increase the viscosity of the intestinal contents, reducing the action of proteolytic enzymes, and consequently causing endogenous nitrogen losses [49]. Another possible explanation is that lignin is a substance of the insoluble fraction of fiber, and its binding with proteins makes them unavailable for animal absorption [37].

For MMDC, the value found for CPM in this study was higher than the value found by [50], while the variety *Gigante* cactus pear meal responded inferiorly, but very close to the value found of digestibility coefficient of 27.66 and 21.42% for young and adult Label Rouge birds, respectively, fed with feed based on corn and soybean meal. Cactus pear is considered a good source of minerals, regardless of the species (*Opuntia* and *Nopalea*), with the highest concentrations found for Ca, K, Mg and P and the lowest for Cu, Fe, Sr and Zn [51,52]. However, cacti possess the antinutritional factor calcium oxalate, which binds to calcium and possibly other minerals in a nutritionally unavailable form, thus interfering with the bioavailability of calcium for animal absorption [37,53]. The researchers [54], observed that the morphology of calcium oxalate crystals was different, since the crystals were larger (ranging from 30 to 100 μm) and more abundant in fresh cladode tissues of the three *Opuntia* *ficus-indica* cultivars (argelina, morado e gymno-carpo) than in *Opuntia* robusta, which were smaller (ranging from 6 μm to 35 μm), more rounded, very sparse and observed mainly near the epidermis. This caused a reduction in calcium concentration in *Opuntia* robusta. Possibly,

the lower mineral matter digestibility coefficient of Gigante cactus pear meal (19.2%) may be associated with calcium oxalate crystals.

Diets containing levels of CPM did not compromise the variables of productive performance, despite mainly the presence of NSPs and oxalic acid. In the scientific literature there are no studies available on the use of cactus pear meal for laying hens, but many studies are found with good alternatives to corn for these birds, but with problems that limit their use. Rice bran, which is alternative to corn, has in its composition a high percentage of phytic acid and NSPs [55], making it a similar feed to cactus pear meal due to its chemical composition and presence of antinutritional factors. Knowing about the presence of the antinutritional factors in rice bran, [56] tested the inclusion of rice bran in laying hens' feed and found that it had no significant effect on egg production, feed intake, feed conversion and egg mass, as did the present study.

Yolk percentage, shell percentage, shell thickness, specific gravity, Haugh Unit, yolk strength and yolk brightness values had no significant effects from the increasing levels of CPM. Cactus pear meal has a high concentration of non-fiber carbohydrates [12], which makes it a good alternative source to corn, but on the other hand there is limitation of use due to the concentration of NSPs [6]. There are no reports in the scientific environment of its use in laying hens, so it is acceptable to compare results with similar feedstuffs in terms of energy and fiber (NSPs). Wheat bran is widely used for laying hens due to its availability and energy, but it is limited due to the amount of NSPs. They [57] found that hens responded similarly to the present study, that is, there was no significant effect of adding 3 and 6% wheat bran and beet pulp in the diets of 90-week-old laying hens on egg shape index, yolk percentage, shell percentage, shell thickness, Haugh unit, and specific gravity.

Eggs from birds that received CPM levels showed lower yolk diameter values, while birds fed corn and soybean meal-based diet had larger yolk diameters, but no studies with similar feeds were found for this trait. Yolk diameter is an important variable, since it is directly related to the reactions that occur in the albumen, where the water from the albumen crosses the yolk membrane by osmosis and is retained in the yolk. Excess water in the yolk determines the increase of its volume, leading to the weakening of the yolk membrane. This makes the yolk appear larger and flattened when the egg is observed on a flat surface after it is broken [58].

The percent of albumen increased according to the increasing use of CPM levels. The response of the albumen percentage had an opposite effect to the yolk percentage, mainly when using 9% CPM, because they are inversely proportional, that is, as the albumen percentage increased, the yolk percentage decreased. Presumably this increase in albumen percentage must be related to the linoleic acid in the birds' diets (Table II). The experimental diets were formulated to contain the same metabolizable energy, so as the level of cactus pear increased it was necessary to increase the amount of soybean oil in the feed to standardize the metabolizable energy. Soybean oil has a reasonable amount of linolenic acid [59], and this acid promotes increased concentrations of estrogen, which is important in controlling egg weight since dietary fats influence egg weight [60,61]. The authors [60] found that diets with supplemental fat and linoleic acid increased albumen weight of eggs of Isabrow hens from the 22nd to 65th week of age.

Alternative feeds to corn are well explored to reduce the cost of poultry production. Understanding the importance of exploring the effects of these feeds on egg quality, [62] evaluated a combination of alternative ingredients and found that the percentage of albumen was higher in group 4 (64.06) than in the other groups (1 - 63.24, 2 - 63.27 and 3 - 63.56), these values are close to those found in the present study.

The shell strength decreased as the level of CPM increased. Hens fed 9% CPM had lower shell strength when compared to the control feed. This reduction may be due to the effect of oxalic acid present in the cactus pear, since it is an organic compound that binds to calcium or other minerals in an unavailable nutritional form, affecting the availability for absorption by the animal [37,45] thus causing deficiency of important minerals for the formation of the shell, since about 94-95% of the dry eggshell is composed of calcium carbonate (CaCO_3).

For yolk coloration, brightness had no significant effect among the experimental diets, but hens fed with 9% CPM had significantly lower values in the red to green region and coloration in the

yellow to blue range. However, the intensity of the yolk color was higher in the control diet, which may be due to the reduced amount of corn in the experimental diets 3, 6, and 9%. A possible reason for this result is that corn is the ingredient source of carotenoids in poultry feeds, and these carotenoids are classified into xanthophylls and carotenes [63,64] added 15% almond shell in the feed of laying hens and found decrease in the values of a (greener) and b (less yellow) in yolk coloration.

The method of texture profile analysis is based on compressing the food for at least two times, simulating the action of two bites on the food. There was no significant effect on Conversion Indicator, which deals with changes suffered (weight increase or reduction) by the cooking process. The hardness property, which is the force required to achieve a deformation of the sample, had no significant effect. Regarding the cohesiveness property, there was linear increase according to the levels of CPM used. Cohesivity is defined as the degree to which a material is deformed before it breaks (physical) or between the teeth before it breaks (sensory) [65]. Probably this significant effect for cohesiveness is related to the amount of fat present in the yolk (Table VIII) since [66] reported that fat in the yolk increases cohesiveness. No significant effect was observed on the elasticity property, which is defined as the degree to which the deformed material returns to its original condition after a force was applied (physical) or pressed between the teeth (sensory). No effects were found for the parameter gumminess either. This is a parameter defined as the energy required to disintegrate a food to a swallowable state. Regarding chewability, which is the number of chews required, at a constant force, for the food to be swallowed [66] there was no significant effect either.

The cooked yolk coloration parameters a^* and b^* were affected by CPM levels, since as the level of CPM increased, the yolk color reduced. The reduction in yolk color intensity may be related to the presence of natural pigments (lutein, zeaxanthin, and β -carotene) [67]. Possibly this variation is because corn is the main carotenoid source in poultry feed [63], which means that the presence of pigments is higher in corn than in cactus pear [68] showed that the average contents of lutein, zeaxanthin, and beta-carotene, in green corn kernels is 0.71, 9.85, and 0.88 $\mu\text{g/g}$ in the fresh sample, respectively.

The mineral matter of eggs from hens fed with CPM reduced, however dry matter and crude protein did not differ between treatments. This possibly occurred because cactus pear has the antinutritional factor calcium oxalate, which is an organic compound that binds to calcium or other minerals in an unavailable nutritional form, affecting availability for animal absorption [37,45].

According to [69], chromatographic analyses of total lipids extracted from cactus pear cladodes show that palmitic acid (C16:0), oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3) contribute in 13.87, 11.16, 34.87 and 32.83% of the total fatty acid content, respectively. These four fatty acids therefore represent over 90% of the total fatty acids with linoleic and linolenic acids being the main polyunsaturated ones, totaling 67.7%.

The saturated fatty acids identified were myristic, palmitic, and stearic. Myristic and palmitic acids reduced as the level of CPM increased, however, stearic acid behaved inversely proportional to myristic and palmitic, which increase in the yolk as the level of CPM increased in the diet.

The monounsaturated fatty acids identified were myristoylic, palmitoleic, oleic, and vaccenic. All monounsaturated fatty acids showed higher concentration in the yolk of eggs from hens fed with the control diet (0% CPM) and reduced as the level of CPM increased. Regarding the oleic acid, a possible explanation for the reduction is the presence of soybean oil in the feed, since in the study of [59] the incorporation of soybean oil reduced oleic acid in yolks from chickens fed with corn.

The polyunsaturated fatty acids linoleic and α -linolenic increased as the level of CPM increased, possibly due to the incorporation of soybean oil in the feed. As the level of CPM increased, the amount of soybean oil also increased. Soybean oil is rich in linoleic acid and has a fair amount of linolenic acid. The inclusion of soybean oil in the diet increased the linoleic and linolenic acid contents and consequently increased the linoleic and α -linolenic fatty acids in the yolk [59]. Linoleic acid promotes increased estrogen concentrations and thus stimulates protein synthesis in the oviduct, causing greater protein deposition in the albumen, resulting in a heavier egg [61]. In addition, linoleic acid has long been accepted as having a hypocholesterolemia effect and inhibitory properties against metastatic colon cancer cells. Omega-3 linolenic acid is known to be beneficial to health,

cardiovascular disease, inflammatory conditions, autoimmune disorders, and diabetes [69]. Arachidonic acid which is the precursor of linoleic acid was detected in the yolks and the lowest concentration was found in eggs from hens receiving the control diet. As the CPM level increased in the diet, the concentration of this fatty acid also increased.

The cholesterol content in the yolk increased linearly with the use of CPM (TC= 397.4 + 20.37CPM, $R^2=0.77$). This can be explained by the increase in the polyunsaturated fatty acids linoleic and α -linolenic. The lipid composition of egg yolk can be altered, especially regarding the fatty acid profile, including the content of n-3 polyunsaturated fatty acids (PUFA) [70]. The cholesterol content in the yolk has become an important issue for consumers, as cholesterol is synthesized by the human body and consumers have been advised to avoid dietary cholesterol intake to prevent chronic diseases, including coronary heart disease. More recently, it has been determined that exogenous cholesterol actually represents a very small amount of hematic cholesterol [71].

Although feed intake was higher for hens receiving the diet with 6% CPM, the price of feed was higher in the 9% CPM diet in comparison to the others, which may be due to the increase in soybean oil in the diets to keep them isoenergetic. Consequently, feeding cost was also higher for the diet containing 6% CPM, due to the higher feed intake. Egg dozen production (dozen/bird) was higher when hens consumed diets containing 6% CPM. A plausible explanation is that with 6% of cactus pear meal in the diet there was higher feed intake, consequently more money was spent to produce the 6% CPM diet. In contrast, the ratio of Feeding Cost/Egg Dozen, R\$/dozen was higher for the control diet, while for the diet with 6% CPM the feeding cost to produce a dozen eggs was higher than the diets with 3% and 9%, respectively.

5. Conclusions

Considering the results, it is possible to use 9% of cactus pear meal in the diet of laying hens in semi-intensive system with no prejudice to the performance parameters and external and internal quality of eggs. When using 3% of cactus pear meal a higher economic return was obtained.

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