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## Passion Fruit Seed (*Passiflora edulis*) in the Diets of Meat-type Quails

### ABSTRACT

The study aimed to determine the chemical composition and energetic value of passion fruit seed (PFS) and evaluate the inclusion of PFS in quail diets regarding performance, carcass characteristics, litter quality, meat lipid stability, and economic viability. In the metabolism assay, 108 21-day-old quails were distributed in a completely randomized design, with one reference diet and two test diets substituting 20% and 40% of the reference diet with PFS, and 6 replications of 6 quails. The AMEn values determined were 4,214.00 kcal/kg DM and 4,127.33 kcal/kg DM for 20% and 40% PFS, respectively. In the performance assay, 432 quails aged 7 to 42 days were distributed in a completely randomized design with inclusion levels of 0, 5, 10, 15, 20, and 25% of PFS, with 6 replications of 12 quails. The results show that feed conversion increased starting at a PFS inclusion of 15%. A linear increase was observed in the percentage of gizzards from quails fed with 25% PFS, along with a linear effect on economic variables starting from a 20% inclusion. It was concluded that PFS can be used at a level of up to 15% in the diets of meat quails aged 7 to 42 days.

### INTRODUCTION

In meat-type quail (*Coturnix coturnix coturnix*) production, the traditionally used feedstuffs are corn and soybean meal. Although presenting good performance, can increase feeding costs due to price and supply fluctuations in these products. Increased production of fruit-processing residues in agro-industries has spurred interest in using agricultural byproducts as animal feed (Gomes *et al.*, 2020).

In this sense, the processing industries of yellow passion fruit (*Passiflora edulis f. flavicarpa*) produce a significant amount of waste in the form of peels and seeds, which account for approximately 60% of the fruit. This waste is generated during the production of juices and pulps and results in tons of solid residues. This not only increases operating costs for the agroindustry but also poses an environmental risk (Coelho *et al.*, 2011).

Passion fruit seeds represent about 12% of the total weight of the fruit and have 8 to 12% crude protein, NDF (50.22%), ADF (43.71%), lignin (5.77%), hemicellulose (6.51%), cellulose (37.05%) and pectin (18.34%), minerals such as sodium, magnesium, and phosphorus with 2.98; 1.54 and 1.25 mg/g, respectively, and a content of 28 to 30% oil, in addition to a high content of phenolic compounds, with possible antioxidant and antibiotic action (Lousada Junior *et al.*, 2006; Malacrida & Jorge, 2012; López-Vargas *et al.*, 2013; Fachinello *et al.*, 2016).

The use of passion fruit seed has been proposed by several authors who point out the potential of this ingredient to be used in animal



feeding (Perondi et al., 2014; Fachinello et al., 2016; Zanneti et al., 2017; Ferreira et al., 2021), making it necessary to estimate the optimal inclusion level and to evaluate the feasibility of using it in meat quail feed.

Given the above, the objective was to determine the energetic value of passion fruit seeds and assess the effects of their addition to meat quail diets on the digestibility of nutrients, as well as on performance, carcass characteristics, litter quality, meat lipid stability, and economic viability.

## MATERIALS AND METHODS

The first part of the project has already been published, and a part of the experimental method has been described in Silva *et al.* (2022).

The experiments were conducted in a manner that avoided unnecessary discomfort to the animals through proper management, laboratory techniques, and experimental procedures. Committee of the Federal University of Ceará under protocol nº 8752160418.

The passion fruit seeds were brought in their wet form from a fruit-pulp agroindustry. They were then washed in running water and subjected to manual friction movements on nylon mesh to help remove the aryl and mucilage. The seeds were then exposed to the sun on a plastic canvas, being rotated four times a day for four days. After drying, they were stored in closed plastic bags at room temperature until required for use in the diets.

The passion fruit seeds were ground using a knife-type mill with a 2.5-mm sieve to determine their chemical composition and metabolizable energy. Samples of the seeds were sent to the Animal Nutrition Laboratory (Federal University of Ceará, Fortaleza, Brazil) to determine their dry matter (DM), nitrogen (N), ether extract (EE), mineral matter (MM), according to Silva & Queiroz (2002), neutral detergent fiber (NDF) and acid detergent fiber (FDA) contents according to the methodology of Van Soest *et al.* (1991). The samples were also tested for gross energy using an IKA C 200an calorimetric pump. The determination of concentrations of the minerals, calcium, phosphorus, potassium, sodium, magnesium, zinc, copper, and iron in the samples of the PFS, was carried out according to the methodology described by Silva *et al.* (2009), and the concentration determined by optical emission spectrophotometry with inductively coupled plasma (ICP-OES), using Perkin Elmer simultaneous spectrometer (Optima 4300DV).

In the metabolism assay, 108 European quails of 21 days old were used for a total of eight days. The first four days were reserved for. At the same time, the remaining four were used to collect excreta, while the remaining four were acclimated to the experimental conditions. The traditional method of total excreta collection was employed for the latter. The quails were handled according to technical recommendations for the period from 1 to 20 days of age. At 21 days, they were weighed and selected based on the initial average weight ( $0.130 \text{ kg} \pm 0.005$ ), as described by Sakomura & Rostagno (2007), to carry out the metabolism assay.

The quails were kept in batteries of metallic cages, distributed in a completely randomized design with 3 treatments and 6 replications of 12 quails per experimental unit. The treatments consisted of a reference diet based on corn and soybean meal (Table 1), formulated to meet the nutritional levels recommended by Silva & Costa (2009), and two test diets. The test diets were made by substituting 20% and 40% of the reference diet with passion fruit seed (PFS).

**Table 1** – Proximate composition and calculated nutritional levels of the reference diet.

| Ingredients                                             | %     |
|---------------------------------------------------------|-------|
| Corn, grain                                             | 53.88 |
| Soybean meal                                            | 40.92 |
| Soybean oil                                             | 2.17  |
| Calcitic limestone                                      | 1.22  |
| Dicalcium phosphate                                     | 0.88  |
| Common salt                                             | 0.36  |
| Mineral and vitamin supplement                          | 0.20  |
| DL – methionine                                         | 0.24  |
| Choline chloride                                        | 0.05  |
| L- lysine HCl                                           | 0.04  |
| Anticoccidial                                           | 0.05  |
| <b>Calculated nutritional and energetic composition</b> |       |
| Metabolizable energy (kcal/kg)                          | 2,950 |
| Crude protein (%)                                       | 23.00 |
| Potassium (%)                                           | 0.23  |
| Calcium (%)                                             | 0.75  |
| Available phosphorus (%)                                | 0.29  |
| Sodium (%)                                              | 0.16  |
| Chlorine (%)                                            | 0.26  |
| Digestible lysine (%)                                   | 1.17  |
| Digestible methionine (%)                               | 0.54  |
| Digestible methionine + cystine (%)                     | 0.85  |
| Digestible threonine (%)                                | 0.78  |
| Digestible tryptophan (%)                               | 0.26  |
| Digestible valine                                       | 0.96  |

<sup>1</sup> Composition per kg of product: Iron – 50.00 g; Copper – 12.00 g; Manganese – 60.00 g; Zinc – 50.00 g; Iodine – 1,000.00 mg; Selenium – 400.00 mg; Vit. A – 20,000,000.00 IU; Vit. D3 – 5,000,000.00 IU; Vit. E – 100,000.00 IU; Vit. K3 – 6,000.00 mg; Vit. B1 – 7,000.00 mg; Vit. B2 – 15.00 g; Niacin – 80.00 g; Pantothenic acid – 30.00 g; Vit. B6 – 8,000.00 mg; Folic acid – 4,000.00 mg; Biotin – 200.00 mg; Vit. B12 – 36,000.00 mg.



During the experiment, fresh water and feed were provided *ad libitum*, with feeders being refilled twice daily to prevent waste. A program with 24 hours of daily light (natural + artificial) was adopted, using 40-watt fluorescent lamps, to stimulate feed intake.

The diets were weighed at the start and end of the collection period to determine the amount consumed. To collect the excreta, individual aluminum trays were used beneath the cages in each experimental plot. These trays were lined with plastic to prevent the sample.

To identify the excreta for collection, 1% ferric oxide was added to the diets as a marker to signal the beginning and the end of the collections. Excreta were collected twice daily, in the early morning and late afternoon, placed in plastic bags, weighed, replicated, and frozen at -20 °C until the end of the experimental period.

To analyze the excreta samples, the material was thawed at room temperature, and a single sample was analyzed. The samples were then pre-dried in a forced ventilation oven at 55 °C for 72 hours. After drying, the samples were ground in a knife-type mill using a 16-mesh sieve with a 1mm sieve for further analysis.

The excreta and experimental diets samples were sent to the Animal Nutrition Laboratory (Federal University of Ceará, Fortaleza, Brazil) for the determination of dry matter and nitrogen, based on the methodology described by Silva & Queiroz (2002). The gross energy was determined in a calorimetric pump model, IKA C 200. For the calculations of AMEn, NMC, and GEMC, laboratory data were used to apply the equations proposed by Matterson *et al.* (1965).

For the performance assay, 432 European quails were used. These quails ranged from 7 to 42 days old and were housed in a conventional masonry shed that was 9.5 m wide × 9 m long. The quails were distributed among 36 boxes, each measuring 0.60 × 0.60 m. Each box was equipped with a tubular-type feeder and a pressure cup-type drinker.

In the first week of age, from 1 to 7 days, the quails were housed in a protection circle with tray-type feeders, pressure cup-type drinkers, and hoods used as heat sources to keep the quails warm. All quails received feed and water at will. At 7 days of age, they were selected based on the mean body weight of the consignment (38.6 g ± 0.67 g) and distributed among the plots according to the recommendations of Sakomura & Rostagno (2007). A completely randomized design was used with 6 treatments: a reference diet and 5 others with increasing levels of

passion fruit seed inclusion (5, 10, 15, 20, and 25%), with 6 replications of 12 quails per experimental unit. The experimental diets (Table 2) were formulated to be iso-nutritive and iso-caloric, according to the requirements proposed by Silva & Costa (2009).

**Table 2** – Proximate composition and calculated nutritional levels of the experimental diets containing passion fruit seed for meat-type quails.

| Ingredients (%)                                         | Levels of inclusion of passion fruit seed |       |       |       |       |       |
|---------------------------------------------------------|-------------------------------------------|-------|-------|-------|-------|-------|
|                                                         | 0%                                        | 5%    | 10%   | 15%   | 20%   | 25%   |
| Corn, grain                                             | 53.88                                     | 50.05 | 46.23 | 42.40 | 38.00 | 32.42 |
| Soybean meal                                            | 40.92                                     | 40.33 | 39.75 | 39.15 | 38.69 | 38.41 |
| Passion fruit seed                                      | 0.00                                      | 5.00  | 10.00 | 15.00 | 20.00 | 25.00 |
| Soybean oil                                             | 2.17                                      | 1.58  | 0.99  | 0.39  | 0.00  | 0.00  |
| Calcitic limestone                                      | 1.22                                      | 1.22  | 1.23  | 1.23  | 1.24  | 1.24  |
| Dicalcium phosphate                                     | 0.87                                      | 0.87  | 0.85  | 0.84  | 0.82  | 0.81  |
| Common salt                                             | 0.36                                      | 0.36  | 0.36  | 0.36  | 0.36  | 0.37  |
| DL – methionine                                         | 0.24                                      | 0.24  | 0.24  | 0.24  | 0.24  | 0.25  |
| L – lysine HCl                                          | 0.04                                      | 0.05  | 0.05  | 0.09  | 0.07  | 0.07  |
| Mineral supplement <sup>1</sup>                         | 0.05                                      | 0.05  | 0.05  | 0.05  | 0.05  | 0.05  |
| Vitamin supplement <sup>2</sup>                         | 0.15                                      | 0.15  | 0.15  | 0.15  | 0.15  | 0.15  |
| Anticoccidial                                           | 0.05                                      | 0.05  | 0.05  | 0.05  | 0.05  | 0.05  |
| Choline chloride                                        | 0.05                                      | 0.05  | 0.05  | 0.05  | 0.05  | 0.05  |
| Washed sand (inert)                                     | 0.00                                      | 0.00  | 0.00  | 0.00  | 0.28  | 1.13  |
| <b>Calculated Nutritional and Energetic Composition</b> |                                           |       |       |       |       |       |
| Metabolizable energy (kcal/kg)                          | 2,950                                     | 2,950 | 2,950 | 2,950 | 2,950 | 2,950 |
| Crude protein (%)                                       | 23.00                                     | 23.00 | 23.00 | 23.00 | 23.00 | 23.00 |
| Calcium (%)                                             | 0.75                                      | 0.75  | 0.75  | 0.75  | 0.75  | 0.75  |
| Available phosphorus (%)                                | 0.29                                      | 0.29  | 0.29  | 0.29  | 0.29  | 0.29  |
| Sodium (%)                                              | 0.16                                      | 0.16  | 0.16  | 0.16  | 0.16  | 0.16  |
| Chlorine (%)                                            | 0.26                                      | 0.26  | 0.26  | 0.26  | 0.25  | 0.25  |
| Digestible lysine (%)                                   | 1.17                                      | 1.15  | 1.13  | 1.12  | 1.10  | 1.08  |
| Digestible methionine (%)                               | 0.54                                      | 0.53  | 0.52  | 0.52  | 0.51  | 0.50  |
| Digestible methionine + cystine (%)                     | 0.85                                      | 0.84  | 0.82  | 0.80  | 0.78  | 0.76  |
| Potassium (%)                                           | 0.23                                      | 0.22  | 0.20  | 0.19  | 0.18  | 0.16  |
| Digestible threonine (%)                                | 0.78                                      | 0.76  | 0.74  | 0.72  | 0.70  | 0.68  |
| Digestible tryptophan (%)                               | 0.26                                      | 0.25  | 0.25  | 0.24  | 0.24  | 0.23  |
| Digestible valine (%)                                   | 0.96                                      | 0.94  | 0.91  | 0.89  | 0.87  | 0.84  |

<sup>1</sup> Composition per Kg of product: Iron – 100,000.00 mg; Copper – 20.00 g; Manganese – 130,000.00 mg; Zinc – 130,000.10 mg; Iodine – 2,000.00 mg; <sup>2</sup> Composition per Kg of product: Vit. A – 9,000,000.00 IU; Vit. D3 – 2,500,000.00 IU; Vit. E – 20,000.00 mg; Vit. K3 – 2,500.00 mg; Vit. B1 – 2,000.00 mg; Vit. B2 – 6,000.00 mg; Vit. B12 – 15.00 mg; Niacin – 35,000.00 mg; Pantothenic acid – 12,000.00 mg; Vit. B6 – 8,000.00 mg; Folic acid – 1,500.00 mg; Selenium – 250.00 mg; Biotin – 100.00 mg.

The chemical composition values of feeds indicated by Rostagno *et al.* (2017) were used as a reference, except for the passion fruit seed, for which it was considered the apparent metabolizable energy value corrected by the nitrogen balance with levels of 20% of substitution by passion fruit seed obtained in the previous experiment and values of chemical-bromatological and mineral composition (Table 2), based on the methodology described by Silva *et al.* (2009). The concentrations of the minerals calcium, potassium, sodium, magnesium, zinc, copper, and iron



were determined by optical emission spectrophotometry with inductively coupled plasma (ICP-OES), using a Perkin Elmer simultaneous spectrometer (Optima 4300DV).

A program of 24 hours of daily light (natural + artificial) was adopted throughout the whole experimental period. Fluorescent lamps of 40 watts were used, distributed at a height of 2.4 m from the floor, so that all quails received light evenly. The temperature and relative humidity of the air inside the shed were measured daily throughout the experimental period by three thermohygrometers distributed in the main areas of the shed, with readings taken twice a day (8 am and 4 pm). The average minimum and maximum room temperatures, and the air relative humidity recorded inside the shed during the experimental period in the performance assay were 28.0, 32 °C, and 63%, respectively.

Nutrient digestibility, meat quality, and litter quality.

To evaluate the effect of the inclusion of passion fruit seed on the metabolism of dietary nutrients, total excreta collection was carried out from 21 to 28 days of age, with three days of adaptation and four days of collection. At 21 days old, six quails from each experimental plot were housed in batteries of metal cages, following the same experimental design, with aluminum trays lined with plastic, individualized by the experimental plot. At the beginning and at the end of the excreta collection period, the quails received a diet containing 1% ferric oxide to signal the beginning and end of the collections.

Excreta collections were performed twice a day, in the early morning at 8 am and in the late afternoon at 4 pm. At the end of each collection, the excreta were packed in plastic bags, duly identified by replication, and frozen until the end of the experimental period to determine the excreta produced. The excreta were then thawed at room temperature, weighed, and homogenized, then pre-dried in a forced-ventilation oven at 55 °C for 72 hours. Then, the samples were ground in a knife mill, with a 16-mesh 1mm sieves, and sent with samples of the experimental diets to the Animal Nutrition Laboratory (Federal University of Ceará, Fortaleza, Brazil) for the determination of dry matter, ashes, and nitrogen, following the methodology described by Silva & Queiroz (2002). The gross energy was determined (IKA C 200).

For performance evaluation, quails and diets were weighed at the beginning of the experiment (7 days old) and at the end of the experimental period (42

days old), to obtain feed intake, weight gain, and feed conversion ratio. Feed intake was obtained by the difference in the amount of feed provided at the beginning of the experiment and the amount of leftovers at the end of the experimental period for each plot. Weight gain was calculated by the mean weight difference of the plot at 7 and 42 days old. The feed conversion ratio was calculated as the ratio of feed intake to weight gain for each plot. At the end, the variables were corrected for mortality.

For carcass evaluation, at 42 days of age and after a 6-hour prior fast, a male and a female from each experimental unit were selected according to the average weight of the plot, desensitized, and slaughtered by bleeding, scalded, plucked, and eviscerated. Then, the head, neck, and feet were removed, and the hot carcass was weighed to determine the carcass yield using the weight of the fasting quail. Subsequently, the cuts were made, the parts were separated, and the entire breast, thigh + drumstick, abdominal fat, liver, and gizzard were weighed to calculate the yield of the cuts. The yields of breast, drumstick + thigh, and abdominal fat were calculated as percentages of the hot carcass weight. The relative weight of the liver and gizzard was obtained concerning the weight of the organs by the weight of the fasting quails. After weighing, the drumsticks + thighs and back of the two quails were ground, divided into two subsamples, identified, frozen in liquid nitrogen at -80 °C, and kept refrigerated (-18 °C), until the moment of lipid oxidation analysis, one with one day of storage and the other with 60 days of storage.

Lipid stability was evaluated after sample storage by measuring the concentration of thiobarbituric acid-reactive substances (TBARS). The calibration curve and sample preparation for the determination of meat lipid oxidation (TBARS) were performed using the liquid acid extraction method, as described by Kang *et al.* (2001). In a 15 mL tube, approximately 2 g of sample was weighed and homogenized with 6.75 mL of perchloric acid (3.86%) and 18.75 µL of BHT (4.5%). Then, 18 mL of perchloric acid (3.86%) was added, and the contents were homogenized in a Terrutec shredder (Tecnal, Piracicaba, SP) at high speed for 15 seconds. The homogenate was filtered, and 0.75mL of this solution was transferred to test tubes with 0.75mL of 2-thiobarbituric acid (20 mM). The tubes were heated in a boiling water bath for 30 minutes. After cooling to room temperature, the spectrophotometer was read at 531nm. The blank used was prepared with 0.75mL of



perchloric acid and 0.75mL of the TBA solution. The number of TBARS in the sample was expressed as µg of malonaldehyde per g of the sample.

At the end of the experimental period, the litter quality was also assessed, with the determination of pH, humidity, temperature, and ammonia concentration in the litter. The temperature of the litter was monitored with a surface thermometer at three different locations in each experimental unit, avoiding areas below the feeders and drinkers. Measurements were carried out at 4 pm on the last day of the experiment. To evaluate the pH and humidity of the bedding, a litter sample from each experimental unit was prepared and collected on the last day of the experiment by taking three subsamples from three different spots within each plot, avoiding areas near and under feeders and drinkers. Subsequently, the samples were homogenized and hermetically packed. To determine the pH, 30g of macerated sample was placed in a beaker, followed by the addition of 250 mL of deionized water. The sample was stirred for 5 minutes. Then, the sample was left to rest for 30 minutes before reading on the pH meter. To determine the moisture content, the methodology proposed by Silva and Queiroz (2002) was used. The concentration of volatilized ammonia was determined according to the adaptation of the methodology proposed by Oliveira *et al.* (2004). 100 g of poultry litter was placed in a plastic container. A lid, and a universal collection cup with a capacity of 50 ml containing 10 ml of boric acid (2%) were placed on this sample to capture the volatilized ammonia inside the container. After adding the sample and the universal collection cup, the plastic container was closed and sealed with adhesive tape. The litter samples were kept in the container for 24 hours. Subsequently, the boric acid was titrated with 0.05N sulfuric acid and the amount of volatilized ammonia was determined using the equation:  $A = V \times N \times (17 / P)$ , with  $A$  being the amount of volatilized ammonia (mg/g);  $V$ , the volume of sulfuric acid used in the titration (ml);  $N$ , the normality of sulfuric acid; 17 the molecular weight of ammonia and  $P$ , the weight of the litter sample (g).

To determine the economic viability of including passion fruit seed in the diet, the ration cost was calculated based on ingredient prices in the city of Fortaleza, CE, during the experimental period. The cost of the ration per kilogram of body weight gain was calculated according to the equation proposed by Bellaver *et al.* (1985), considering  $Y_i = (Q_i \times P_i) / G_i$ , where  $Y_i$ =amount spent on ration per kilogram of

body weight in the  $i^{th}$  treatment;  $P_i$ =price of kilogram of the ration used in the  $i^{th}$  treatment;  $Q_i$ =amount of ration consumed in the  $i^{th}$  treatment and  $G_i$ =weight gain in the  $i^{th}$  treatment. The economic efficiency index (EEI) and the cost index (CI) proposed by Fialho *et al.* (1992) were calculated as:  $EEI = (MCE_i / CTE_i) \times 100$  and  $CI = (CTE_i / MCE_i) \times 100$ , where  $MCE_i$ =the lowest cost per kilogram of gain observed among treatments, and  $CTE_i$ =the cost of treatment  $i$  considered.

The statistical analysis of the data was performed using SAS. The data on apparent metabolizable energy corrected for passion fruit seed were subjected to analysis of variance and an F-test (5%). The data on diet digestibility, performance, carcass characteristics, litter quality, and economic viability were subjected to analysis of variance, and the means were compared using the Dunnett test (5%). The lipid stability data were subjected to analysis of variance using a completely randomized model in a 6 × 2 factorial scheme (6 levels of inclusion of the tested feed and 2 storage times), and the means were compared using the SNK test (5%). All variables were subjected to regression analysis to determine the optimal level of inclusion of passion fruit seed, considering only data from treatments that included the tested feed, excluding the control treatment.

## RESULTS

The results of the chemical, energetic, and mineral composition of the passion fruit seed (PFS) are expressed on an as-fed basis (Table 3).

**Table 3** – Chemical, energetic, and mineral composition of passion fruit seed expressed on an as-fed basis.

| Parameters                  | Passion fruit seed |
|-----------------------------|--------------------|
| Dry matter (%)              | 91.89              |
| Gross energy (kcal/kg)      | 5,258              |
| Crude protein (%)           | 8.56               |
| Ether extract (%)           | 11.98              |
| Neutral detergent fiber (%) | 67.71              |
| Acid detergent fiber (%)    | 62.23              |
| Mineral matter              | 1.00               |
| Calcium (%)                 | 0.07               |
| Phosphorus (%)              | 0.20               |
| Potassium (g/kg)            | 0.25               |
| Sodium (g/kg)               | 0.03               |

<sup>1</sup> Analyses performed in the Animal Nutrition Laboratory and Soils Laboratory of Embrapa.

In the determination of the metabolizable energy, there was no significant difference between the



values of apparent metabolizable energy corrected by the nitrogen balance (AMEn) of the passion fruit seed determined with the levels of 20 and 40% of substitution of the reference diet (Table 4).

**Table 4** – Mean values of corrected apparent metabolizable energy (AMEn) of passion fruit seed determined using meat quails, expressed on a dry matter basis.

| Variable          | Levels of inclusion |          | Mean     | CV <sup>1</sup> | p-value |
|-------------------|---------------------|----------|----------|-----------------|---------|
|                   | 20%                 | 40%      |          |                 |         |
| AMEn (kcal/kg DM) | 4,214.00            | 4,127.33 | 4,170.66 | 2.99            | 0.2501  |

<sup>1</sup> CV=Coefficient of variation.

According to the results obtained through the metabolism assay (Table 5), regarding the control diet (0%), the diets containing 15, 20 and 25% of inclusion of the passion fruit seed showed lower values of metabolizability coefficients of the dry matter (DMMC) and gross energy (GEMC), while for the apparent metabolizable energy (AME) and apparent metabolizable energy corrected by the nitrogen balance on dry matter basis (AMEn) the values were lower in the diets with inclusion of 20 and 25%. However, there was no significant difference in the nitrogen metabolizability coefficient (NMC).

**Table 5** – Metabolizability coefficients and metabolizable energy values of the diets for meat-type quails containing passion fruit seed.

| Variable         | Level of inclusion (%) |       |       |        |        |        | CV <sup>6</sup> | p-value              |         |           |
|------------------|------------------------|-------|-------|--------|--------|--------|-----------------|----------------------|---------|-----------|
|                  | 0                      | 5     | 10    | 15     | 20     | 25     |                 | Level                | Linear  | Quadratic |
| GEMC (%)         | 87.57                  | 86.30 | 87.06 | 83.01* | 82.06* | 81.45* | 1.72            | <0.0001 <sup>1</sup> | <0.0001 | 0.9073    |
| DMMC (%)         | 85.85                  | 84.67 | 85.70 | 80.96* | 81.25* | 79.52* | 2.27            | <0.0001 <sup>2</sup> | <0.0001 | 0.8892    |
| NMC3 (%)         | 70.42                  | 69.17 | 70.24 | 69.80  | 72.25  | 70.24  | 4.46            | 0.74                 | 0.2739  | 0.661     |
| AME (kcal/kgDM)  | 3,644                  | 3,611 | 3,649 | 3,541  | 3,431* | 3,405* | 1.72            | <0.0001 <sup>3</sup> | <0.0001 | 0.2831    |
| AMEn (kcal/kgDM) | 3,414                  | 3,372 | 3,394 | 3,338  | 3,194* | 3,187* | 1.47            | <0.0001 <sup>4</sup> | <0.0001 | 0.1526    |
| AMEn (kcal/kgAF) | 3,080                  | 3,083 | 3,069 | 3,025  | 2,907* | 2,907* | 1.47            | <0.0001 <sup>5</sup> | <0.0001 | 0.5967    |

<sup>1</sup> GEMC ( $Y=88.38 - 0.293X$ ,  $R^2=0.61$ ), <sup>2</sup>DMMC ( $Y=86.84 - 0.2949X$ ,  $R^2=0.52$ ), <sup>3</sup>AME ( $Y=3,716 - 125.88X$ ,  $R^2=0.63$ ), <sup>4</sup>AMEn DM ( $Y=3,469 - 114.32X$ ,  $R^2=0.65$ ), <sup>5</sup>AMEn AF ( $Y=3,152 - 102.52X$ ,  $R^2=0.69$ ), <sup>6</sup>CV=Coefficient of variation; \*Significant statistical effect by the Dunnett test ( $p<0.05$ ).

In the regression analysis, except for NMC, which was not significantly different, there was a linear decreasing effect on all variables, starting at the 5% inclusion level of passion fruit seed.

For the performance variables (Table 6), there was a significant difference between treatments for feed intake and feed conversion ratio, while weight gain did not differ. Feed intake increased for the quails

fed the levels of 10, 15, 20, and 25% in comparison to those fed the control diet. However, for the feed conversion ratio, differences were observed at levels of 15, 20, and 25%. In the regression analysis, the inclusion of passion fruit seed was associated with a linear increase in feed intake and a linear decrease in the feed conversion ratio.

**Table 6** – Performance of meat-type quails fed diets containing passion fruit seed from 7 to 42 days of age.

| Variable              | Level of inclusion (%) |        |         |         |           |           | CV <sup>2</sup> | p-value              |         |           |
|-----------------------|------------------------|--------|---------|---------|-----------|-----------|-----------------|----------------------|---------|-----------|
|                       | 0                      | 5      | 10      | 15      | 20        | 25        |                 | Level                | Linear  | Quadratic |
| Feed intake (g/quail) | 837.40                 | 919.60 | 928.40* | 930.00* | 1,104.20* | 1,111.00* | 5.00            | <0.0001 <sup>3</sup> | <0.0001 | 0.1213    |
| Weight gain (g/quail) | 225.20                 | 226.00 | 230.00  | 223.00  | 228.00    | 232.40    | 3.10            | 0.3538               | 0.3006  | 0.2990    |
| FCR <sup>1</sup>      | 3.72                   | 4.06   | 4.04    | 4.17*   | 4.84*     | 4.78*     | 5.18            | <0.0001 <sup>4</sup> | <0.0001 | 0.3331    |

\* Statistically different when compared to the control treatment by the Dunnett test ( $p<0.05$ ). <sup>1</sup>Feed conversion ratio. <sup>2</sup>Coefficient of variation. <sup>3</sup>Feed intake ( $Y=831.06 + 11.17X$ ,  $R^2=0.62$ ) and <sup>4</sup>Feed conversion ratio ( $Y=3.71 + 0.05X$ ,  $R^2=0.58$ ).

The results of the carcass characteristics evaluation (Table 7) demonstrated that the carcass yield, the proportion of breast, drumstick + thigh, abdominal fat, and liver did not differ between quails fed the diets containing passion fruit seed in comparison to the control treatment.

However, the inclusion of 25% passion fruit seed

in the diet promoted an increase in the relative weight of the gizzard when compared to the values obtained with the control treatment. In the regression analysis, no significant difference was observed for the carcass characteristics, except for the percentage of gizzard, which showed a linear increase ( $Y=1.88 + 0.01X$ ,  $R^2=0.26$ ) as the passion fruit seed level increased.



**Table 7** – Carcass yield, cuts, and percentage of organs of meat-type quails fed diets containing passion fruit seed from 7 to 42 days of age.

| Variable              | Level of inclusion (%) |        |        |        |        |        | CV <sup>1</sup> | p-value |        |           |
|-----------------------|------------------------|--------|--------|--------|--------|--------|-----------------|---------|--------|-----------|
|                       | 0                      | 5      | 10     | 15     | 20     | 25     |                 | Level   | Linear | Quadratic |
| Final Weight (g)      | 257.20                 | 258.00 | 261.80 | 255.20 | 260.00 | 264.80 | 2.71            | 0.3363  | 0.2617 | 0.2790    |
| Carcass yield (%)     | 78.18                  | 76.13  | 75.93  | 76.03  | 78.65  | 75.98  | 2.40            | 0.0788  | 0.3478 | 0.4209    |
| Breast (%)            | 41.90                  | 40.51  | 42.09  | 41.81  | 41.69  | 41.67  | 3.00            | 0.4349  | 0.2967 | 0.1572    |
| Drumstick + thigh (%) | 22.38                  | 23.09  | 22.51  | 22.91  | 22.82  | 23.17  | 3.79            | 0.6578  | 0.7031 | 0.3600    |
| Abdominal Fat (%)     | 1.09                   | 1.17   | 1.23   | 0.75   | 0.97   | 1.01   | 51.56           | 0.7599  | 0.4169 | 0.4479    |
| Gizzard (%)           | 1.93                   | 1.97   | 2.04   | 2.00   | 2.13   | 2.26*  | 8.03            | 0.0409  | 0.0092 | 0.3286    |
| Liver (%)             | 2.04                   | 2.13   | 1.82   | 1.71   | 2.03   | 1.97   | 23.09           | 0.6952  | 0.8743 | 0.2083    |

\* Significant effect by the Dunnett test ( $p < 0.05$ ); <sup>1</sup> Coefficient of variation

In evaluating the lipid stability of the meat by measuring the concentration of substances reactive to 2-thiobarbituric acid (TBARS), no significant interaction between the tested inclusion levels and storage times was observed (Table 8).

**Table 8** – Lipid oxidation of the meat (breast, drumstick, and thigh) of meat-type quails fed diets containing passion fruit seed.

| Factor                        | TBARS<br>(mg of malonaldehyde/kg) |
|-------------------------------|-----------------------------------|
| <b>Level of Inclusion (%)</b> |                                   |
| 0                             | 2.25                              |
| 5                             | 2.12                              |
| 10                            | 2.23                              |
| 15                            | 1.92                              |
| 20                            | 2.01                              |
| 25                            | 2.02                              |
| <b>Time (Days)</b>            |                                   |
| 0                             | 1.45                              |
| 60                            | 2.74                              |
| Mean                          | 2.09                              |
| Coefficient of Variation (%)  | 15.80                             |
| <b>Analysis of variance</b>   |                                   |
| Level                         | 0.1963                            |
| Storage time                  | <0.0001                           |
| Level x Storage time          | 0.3473                            |
| <b>Regression</b>             |                                   |
| Linear                        | 0.5654                            |
| Quadratic                     | 0.8278                            |

Non-significant statistical effect by the SNK test ( $p > 0.05$ ).

When comparing the treatments that included the passion fruit seed to the control, no significant difference ( $p > 0.05$ ) was observed in TBARS mean values. Also, no linear or quadratic effect was observed in the evaluation of the TBARS values, coming only from the treatments that included passion fruit seed. However, the average TBARS value in meat stored for 60 days was significantly higher than that in meat stored for 1 day.

It was observed that the variables evaluated for litter quality were not influenced by the different levels of passion fruit seed used compared with the control treatment. No effects were also observed in the linear and quadratic regression analyses (Table 9).

Regarding the economic viability (Table 10), there was a significant difference between the averages of feeding costs, in the economic efficiency index (EEI) and cost index (CI) in diets with levels of 20% and 25% when compared to the control treatment, presenting higher cost with feeding and impairment CI and EEI. Based on the regression analysis, the inclusion of passion fruit seed linearly increased the cost of feeding per kg of weight gained ( $Y = 4.87 + 0.0201X$ ,  $R^2 = 0.16$ ) and the CI ( $Y = 100.6408 + 0.4198X$ ,  $R^2 = 0.1716$ ). As a result, EEI linearly reduced ( $Y = 99.6634 - 0.3806X$ ,  $R^2 = 0.1668$ ).

**Table 9** – Quality parameters of the litter of meat-type quails fed diets containing passion fruit seed.

| Variable                   | Level of inclusion (%) |       |       |       |       |       | CV <sup>1</sup> | p-value |        |           |
|----------------------------|------------------------|-------|-------|-------|-------|-------|-----------------|---------|--------|-----------|
|                            | 0                      | 5     | 10    | 15    | 20    | 25    |                 | Level   | Linear | Quadratic |
| Moisture (%)               | 12.17                  | 12.32 | 12.98 | 11.33 | 11.59 | 11.93 | 8.72            | 0.2130  | 0.1840 | 0.5081    |
| pH                         | 8.64                   | 8.38  | 8.21  | 8.35  | 7.50  | 8.16  | 9.00            | 0.2786  | 0.3094 | 0.6184    |
| Volatilized Ammonia (mg/g) | 0.33                   | 0.28  | 0.33  | 0.27  | 0.23  | 0.21  | 41.13           | 0.0757  | 0.0870 | 0.5842    |
| Temperature (°C)           | 32.16                  | 32.43 | 32.77 | 32.20 | 32.32 | 32.19 | 2.53            | 0.8403  | 0.3702 | 0.8305    |

Non-significant statistical effect by the Dunnett test ( $p < 0.05$ ); <sup>1</sup> Coefficient of variation



**Table 10** – Economic evaluation of the inclusion of passion fruit seed in the feeding of meat-type quails.

| Variable                      | Level of inclusion (%) |        |        |        |         |         | CV <sup>1</sup> | p-value |                     |           |
|-------------------------------|------------------------|--------|--------|--------|---------|---------|-----------------|---------|---------------------|-----------|
|                               | 0                      | 5      | 10     | 15     | 20      | 25      |                 | Level   | Linear              | Quadratic |
| Cost of feeding (U\$/kg gain) | 0.99                   | 1.05   | 1.01   | 1.01   | 1.14*   | 1.09*   | 5.25            | 0.0025  | 0.0406 <sup>2</sup> | 0.3369    |
| Economic efficiency index (%) | 100.14                 | 94.78  | 98.59  | 98.14  | 87.35*  | 90.88*  | 5.57            | 0.0053  | 0.0426 <sup>3</sup> | 0.3089    |
| Cost index (%)                | 99.95                  | 105.93 | 101.92 | 102.14 | 114.60* | 110.08* | 5.26            | 0.0025  | 0.0395 <sup>4</sup> | 0.3349    |

<sup>1</sup>Coefficient of variation; <sup>2</sup> $Y=4.87 + 0.0020X$ ,  $R^2=0.16$ ; <sup>3</sup> $Y=99.66 - 0.3800X$ ,  $R^2=0.17$ ; <sup>4</sup> $Y=100.64 + 0.4198X$ ,  $R^2=0.1716$ .

## DISCUSSION

The results of the chemical, energetic, and mineral composition of PFS indicate that the contents of gross energy, protein, ether extract, fibers, and minerals are higher than those found in corn (Rostagno *et al.*, 2017), which is the main energy feedstuff used in poultry feeding.

The results obtained in the study carried out with the PFS, differ from those found by Fachinello *et al.* (2016) who obtained 92.33% dry matter, 11.34% crude protein, 5,569 kcal/kg gross energy, 18.84 ether extract, 50.22% neutral detergent fiber (NDF), 43.71% acid detergent fiber (ADF), and 3.52% of ashes. Similar values were found by Ferreira *et al.* (2021), who observed 65.66% and 62.55% for NDF and ADF, respectively. The calcium and phosphorus contents in the passion fruit seed obtained were 0.07% and 0.20%, respectively, which were similar to those reported by Romo & Nava (2007), which presented 0.06% and 0.27%, respectively, but lower to those obtained by Zanetti *et al.* (2018) of 0.08% calcium (%) and 0.43% total phosphorus, respectively.

Variations in the chemical composition of passion fruit seeds may be linked to the soil type of the region, various cultivation methods, and fertilization. Fertilization is a critical factor affecting the quality of passion fruit and may cause variations in fruit composition (Mendonça *et al.*, 2006). Differences in fruit processing also affect the final quality of the waste (Ferreira *et al.*, 2021).

In the determination of the metabolizable energy, the passion fruit seed AMEn values determined for meat quails were higher than those found by Fachinello *et al.* (2016), who found an AMEn value of 2,939 kcal/kg for Japanese quails at 21 days of age. However, these differences can be justified, mainly, by the variation in energy and fiber content in the composition of passion fruit seeds. The value was also above the one found by Zanetti *et al.* (2018), which was 3,945.41 kcal/kg for broilers, and that is justified by the difference between species.

Due to these results, it is possible to recommend the value of 4,214.00 of AMEn/kg of DM of the passion fruit seed for formulations of the meat quails diets, since the results for the AME values obtained with the substitution of the reference diet by the tested feed in 20% has been recommended for fibrous feeds, using the total excreta collection (Sakomura & Rostagno, 2007).

The increase in fiber content with the inclusion of passion fruit seeds may be associated with a negative effect on nutrient digestibility, reducing metabolization coefficients and metabolizable energy values in diets. For Chau & Huang (2004), the fibrous fraction of the passion fruit seed is made up mostly of insoluble fiber (98.9%), with the soluble fraction represented by 1.13%. Feeds with high content of insoluble fiber can cause shorter time of exposure of the digesta to the digestion processes, since its presence in high concentrations tends to increase the rate of passage of the digesta, resulting in a reduced availability of dietary nutrients (Khajali & Slominski, 2012) and, consequently, in less metabolizable energy.

Although the GEMC decreased with increasing passion fruit seed inclusion levels, from 15% onward, there was no loss in AMEn values. This fact may be associated with the results obtained for NMC, since the value of AMEn will vary depending on the amount of nitrogen retained. The results obtained are similar to those reported by Fachinello *et al.* (2016), who evaluated the substitution of up to 16% of the reference diet with passion fruit seed in the feeding of meat quails and obtained a quadratic effect for AME and AMEn indicating maximum values of these variables with 6.56 and 7.72% of substitution, respectively, and reduction in levels above these.

For the performance variables, voluntary feed intake by quails is mainly regulated by the amount of dietary energy allocated to the organism's metabolic processes, since adjustments in feed intake in response to energy levels reflect quails' attempts to meet their nutritional requirements. Thus, the effects of passion



fruit seed on feed intake can be associated with the need for greater consumption by the quails, since there was a reduction in the metabolizable energy of the diets. As a result, there was an impairment in the feed conversion ratio as the weight gain of the quails was not influenced by the increase in feed consumption.

The obtained results differ, in part, from those reported by Zanetti *et al.* (2018), who, although they did not observe a difference in feed intake and weight gain of broilers fed passion fruit seed with levels of up to 12.5%, found a linear increase in feed conversion ratio at the level 12.5%. Togashi *et al.* (2008) did not observe a difference in feed conversion testing levels of 4 and 8% in the inclusion of passion fruit seed in the feeding of broilers.

With the reduction in the AMEn values of the diets due to the increased inclusion of passion fruit seed, certain changes are observed in the relationships between energy and protein levels, and consequently between energy and amino acid levels. Thus, as established, the magnitude of these changes may influence the deposition of muscle and fat in the carcass, leading to changes in carcass characteristics of meat quails. However, this effect was not confirmed, as the results demonstrate that although the diets' AMEn decreased, the alterations in the previously reported ratios were not sufficient to produce a significant effect on the carcass characteristics of the meat quails.

The results obtained for the variables of carcass characteristics in function of the inclusion of passion fruit seed in the diets of meat quails are similar to those reported in the literature. Barros Júnior *et al.* (2018) did not find a significant difference in the yield of carcass and noble cuts when evaluating the inclusion of passion fruit seeds up to 12% in the diets for female meat quails. Zanetti *et al.* (2018) reported that it is possible to include up to 12.5% of passion fruit seed in the feeding of broilers up to 42 days of age without influencing the yields of carcass, cuts, and abdominal fat. Togashi *et al.* (2008) found no difference between the weights of breast and leg (drumstick + thigh), as well as liver, gizzard, and abdominal fat, using up to 8% of inclusion of passion fruit seed for broilers up to 42 days old.

The greater relative weight of the gizzard can be associated to the fact that the high content of insoluble fiber in the passion fruit seed (Chau & Huang, 2004) increases the dietary fiber content in the diet, especially of this fraction, in a way that the inclusion of the level 25% may have resulted in greater peristalsis stimulus of the gastrointestinal tract (Khajali & Slominski,

2012). The stimulus for greater gizzard activity during the maceration of food particles tends to develop the organ's muscle, culminating in an increase in its weight. The increase in gizzard size is also related to the greater resistance of the fiber to crushing and to the longer time it spends in the gizzard until it reaches the proper size. However, Barros Júnior *et al.* (2018) found no significant difference in the proportion of gizzard when they evaluated the inclusion of passion fruit seeds up to 12% in the diet of female meat quails.

Due to the presence of monounsaturated and polyunsaturated fatty acids in quail meat (Sabow *et al.*, 2022), normally, even immediately frozen after slaughter and kept under freezing (-18 °C), it is prone to lipid oxidation. However, with the influence of passion fruit seed in the diet, two aspects must be considered regarding the oxidation of quail meat: passion fruit seed in the diet. The first is that, in the lipid composition of passion fruit seed oil, stand out linoleic acid (63.98%), oleic acid (19.53%), palmitic acid (11.29%), stearic acid (3.54%), and linolenic acid (0.40%), with linoleic and linolenic acids being more susceptible to lipid oxidation in the meat (Wang *et al.*, 2000) and, therefore, inclusion of higher levels could increase the proportion of unsaturated fatty acids in the meat and, thus, potentiate oxidation. The second one refers to the presence of chemical compounds in the passion fruit seed such as flavonoids, isoflavones, phenolic acids, tocopherols, and polyphenols that, in addition to the antioxidant effect, have a wide range of biological and pharmacological effects such as antibacterial, antiviral, anti-inflammatory, antiallergic and vasodilators (Zeraik *et al.*, 2010) that can contribute to improving the lipid stability of the meat. Therefore, it is possible that the interaction between these factors contributed to the fact that the inclusion of passion fruit seed at higher levels did not influence the lipid oxidation of the quail meat during the storage period.

The results obtained for the lipid oxidation of the quails' meat are similar to those reported by Zanetti *et al.* (2018), who did not observe the effect of the inclusion of passion fruit seed on the lipid oxidation of chicken thigh meat, with only differences between storage times.

Regarding the analyzed quality litter parameters, humidity can influence the appearance of carcass lesions in birds (Qiu & Guo, 2010) and control ammonia volatilization, since its increase promotes greater ammonia release (Dančová *et al.*, 2025). In addition, it increases the risks and contamination by pathogenic agents (Vieira *et al.*, 2015). The moisture



results of this study are below the upper limit reported by Rico-Contreras *et al.* (2017), who state that levels above 25% can cause production losses.

When pH is reduced, ammonia emission decreases, as the growth medium is unfavourable for the survival of the main pathogenic microorganisms (Jie *et al.*, 2022). With pH below 7, ammonia volatilization decreases; therefore, the free H<sup>+</sup> ions increase the ammonium: ammonia ratio, slowing the decomposition of nitrogenous substances (Dančová *et al.*, 2025). The pH values determined for the different treatments evaluated in this research range from 7.50 to 8.64, with no significant effect compared to the control treatment.

The combination of higher litter moisture and elevated environmental temperatures promotes microbial growth and activity, which quickly convert uric acid into NH<sub>3</sub> gas (Garcês *et al.*, 2013). Under ideal production conditions, the litter temperature should be close to the ambient temperature. The litter temperature between treatments ranged from 32.16 °C to 32.77 °C, with no significant difference between the passion fruit seed treatment and the control.

Regarding the concentration of ammonia, the results found in the proposed study were already expected, since they did not show any significant difference between treatments for the parameters of humidity and pH of the litter, these variables being the ones that most influence the quality of air inside the shed (Fogaça *et al.*, 2022).

Regarding the analysis of the economic viability of including passion fruit seeds in quail feed, we can infer that in the making of conventional diets, corn is the most used ingredient as an energy source, however, passion fruit seed can be used instead of corn, as it represents 43% of the average value of this grain, resulting in a lower cost per kg of ration. Even so, as the inclusion of passion fruit seed increases in the diet, economic viability is impaired due to increased feed intake caused by the higher fiber level of the diet. This impact on economic evaluation is significant when the inclusion of passion fruit seed in the diet exceeds 15%, and the indicators of economic viability get significantly impaired when compared to those obtained with the control diet.

## CONCLUSION

According to the technical and economic feasibility parameters, it is concluded that passion fruit seed can be used up to a level of 15% in the feed for meat-type

quails aged 7 to 42 days, without altering the carcass characteristics and the meat lipid stability.

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## AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design. Conceptualization, TFS, ERF, RCN, and TRG; methodology, TFS, ERF, and RCN; validation, TFS, ERF, RCN, and TRG; data collection and analysis, TFS, OFS, MCAM, PJDO, and EOS; investigation, TFS, PJDO, EOS and RCN; writing—original draft preparation, TFS and RCN; writing—review and editing, TRG; visualization, ERF, RCN and TRG; supervision, ERF. All authors have read and agreed to the published version of the manuscript.

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## DATA AVAILABILITY STATEMENT

The data will be available upon request.

## CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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