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Original Article

Phytotherapeutics and Probiotics as an Alternative to the Use of Antimicrobials in the Diets of Laying Quails

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ABSTRACT

This study evaluated the substitution of commercial antibiotics with phytotherapeutics and probiotics in Japanese quail diets during the rearing phase, and their effects on performance, serum metabolites, organ weight and size, and egg quality in the initial laying phase. Conducted over 42 days with 300 quails, starting at 21 days of age and an average weight of 87 ± 3.5 g, the quails were divided into five groups: negative control (no additives), commercial antibiotics, probiotics (BS Gold®), essential oils (Phyto 100®), and natural pollen. The experiment was analyzed using variance analysis and Tukey's test with a 5% significance level. Feed conversion differed significantly in the periods between 28-35 and 35-42 days. Egg quality showed variations, with higher albumen height in the probiotic group, better yolk height and luminosity with pollen, increased luminosity in the control group, and a larger yolk diameter with phytotherapeutics. The findings indicated that essential oils, probiotics, and pollen effectively replaced antibiotics without negatively affecting zootechnical performance, serum metabolites, or organ weight and size. Additionally, egg performance and quality were maintained during the initial laying phase.

INTRODUCTION

In recent years, animal production has undergone substantial changes, particularly regarding the reduction in the use of antibiotics as growth promoters (Diaz Gonzalez *et al*., 2018). In this scenario, a promising perspective emerges for the adoption of alternatives that not only contribute to animal health, but also demonstrate beneficial impacts on animal performance (Mostafa *et al*., 2023), as well as in the mitigation of infections caused by pathogens (Hernandes-Patlan *et al*., 2019).

Many researchers agree that the selective incorporation of certain antimicrobial agents into feed can influence the proportion of resistant bacteria in the gastrointestinal tract (Gastalho *et al*., 2014; Bezerra *et al*., 2017). Phytotherapeutics and probiotics have emerged as representative alternative additives for replacing antibiotics in poultry diets (Carvalho *et al*., 2018; Lima *et al*., 2019; Machado *et al*., 2020).

Phytotherapeutics, including plant extracts, herbs and essential oils, have long been integrated into human diets and are recognized for their health benefits for both humans and animals. Furthermore, these plant extracts, known for their antimicrobial properties, can control and restrict the growth of a variety of pathogenic and non-pathogenic bacterial species in the intestine (Oluwafeni *et al*., 2020).

The underlying hypothesis of this study is that zootechnical performance, serum metabolites, relative weight and the size of reproductive and digestive organs in the rearing phase, as well as the subsequent effects on the performance and quality of eggs of Japanese quails in the initial laying phase, would remain consistent in birds fed diets containing phytotherapeutics or probiotics compared to those receiving diets containing antibiotics as growth promoters.

Thus, the primary objective of this study was to investigate the replacement of commercial antibiotics with commercial phytotherapeutics and probiotics in diets intended for Japanese laying quails (*Coturnix japonica*) during the rearing phase, aiming to understand the impact of these antibiotics on the health and growth of the birds.

MATERIALS AND METHODS

Study location and Ethics statement

The experiment was conducted in the Poultry and Quail Farming Sector of the Faculty of Agricultural Sciences at the Universidade Federal da Grande Dourados (UFGD), lasting for 42 days. It was divided into 2 periods of 21 days each, comprising the rearing phase from 21 to 42 days and the initial laying phase from 42 to 63 days of age. The project was submitted to and approved by the Research Ethics Committee of UFGD (protocol number 16/2020).

The design used was completely randomized, comprising five diets, each with 10 repetitions and 6 quails per experimental unit, totaling 300 birds distributed in a completely randomized design composed of 5 treatments: negative control (without additive inclusion), commercial antibiotic (Bacitracin), probiotic (BS Gold®), a commercial blend of essential oils (Phyto 100®), and natural pollen. The commercial products were acquired from commercial companies and offered in the quantities indicated by the supplier.

Management in the rearing phase

The birds were fed diets formulated for the rearing phase from 21 to 42 days. The lighting program consisted of 24 hours of artificial light until the 15th day of the birds' lives, and subsequently, until 42 days of age, a natural photoperiod was used to prevent the birds from entering sexual maturity prematurely. The diets used were isonutritive, following the recommendations of Rostagno *et al*. (2017), as shown in Table 1.

The quails were housed in parallel battery cages on three tiers. The cage dimensions were of 25 cm width, 35 cm length, and 20 cm height, corresponding to an area of 175 cm²/bird housed, and were equipped with trough feeders and nipple drinkers.

Table 1 – Composition and percentage of experimental diets with different performance-enhancing additives in the rearing phase (21 to 42 days).

Ingredients	Treatments				
	CN	CP	FC	PC	PN
Corn	49.22	49.22	49.22	49.22	49.22
Soybean Meal	43.35	43.35	43.35	43.35	43.35
Soybean oil	2.50	2.50	2.50	2.50	2.50
Inert	2.00	2.00	2.00	2.00	2.00
Bacitracin (15%)	0.00	0.014	0.00	0.00	0.00
Herbal Medicine	0.00	0.00	0.01	0.00	0.00
Probiotic	0.00	0.00	0.00	0.03	0.00
Pollen	0.00	0.00	0.00	0.00	0.06
Limestone	1.22	0.72	0.72	0.72	0.72
Dicalcium Phosphate	0.89	0.89	0.89	0.89	0.89
Salt	0.32	0.32	0.32	0.32	0.32
Dl-methionine	0.16	0.16	0.16	0.16	0.16
L-lysine	0.11	0.11	0.11	0.11	0.11
Mineral Supplement	0.10	0.10	0.10	0.10	0.10
Vitamin Supplement	0.10	0.10	0.10	0.10	0.10
Nutritional Facts					
Metabolizable energy (Kcal/kg)	2900	2900	2900	2900	2900
Crude Protein (%)	24.36	24.36	24.36	24.36	24.36
Digestible lysine (%)	1.09	1.09	1.09	1.09	1.09
Met + Cist (%)	0.74	0.74	0.74	0.74	0.74
Tryptophan (%)	0.18	0.18	0.18	0.18	0.18
Threonine (%)	0.73	0.73	0.73	0.73	0.73
Calcium (%)	1.09	1.09	1.09	1.09	1.09
Phosphorus (%)	0.51	0.51	0.51	0.51	0.51
Sodium (%)	0.20	0.20	0.20	0.20	0.20

CN:negative control. CP: Positive control. FC: Commercial herbal medicine. PC: Commercial probiotic. PN: Natural pollen. The vitamin supplements/kg of diet were as follows: folic acid (Min.) 145.4 mg; pantothenic acid (min.) 5,931.6 mg; choline (min.) 121.8 g; niacin (min.) 12.9 g; selenium (min.) 480.0 mg; vitamin A (min.) 5,000,000.0 IU; vitamin B12 (min.) 6,500.0 mcg; vitamin B2 (min.) 2,000.0 mg; vitamin B6 (min.) 250.0 mg; vitamin D3 (min.) 1,850,000.0 IU; vitamin E (min.) 4,500.0 IU; and vitamin K3 (min.) 918.0 mg. 2Mineral supplement/kg: Copper (min.) 7,000.0 mg; iron (min.) 50.0 g; iodine (min.) 1,500.0 mg; manganese (min.) 67.5 g; zinc (min.) 45.6 g.

The temperature and relative humidity (RH) were monitored daily at 08:00 AM using a digital thermometer hygrometer positioned in the center of the poultry house at the height of the birds' backs. The average maximum and minimum temperatures were 30.6°C and 20.01°C, respectively, while the maximum and minimum humidities were 82.62% and 31.75%, respectively.

Performance evaluation in the rearing phase

The weight gain per bird per day (WG), feed consumption per bird per day (FC), and feed conversion ratio (FCR) were evaluated according to the following methodology:

WG: The average weight gain per bird per day was determined from the weights measured at 21, 28, 35, and 42 days of age, always in the morning.

FC: The average feed consumption was determined by dividing the difference between the feed provided during the phase and the leftover feed weighed at the end of the same phase by the number of birds in the plot. Weighing of the leftovers was also performed on a scale, and the averages were summed to yield the average feed consumption per bird in the plot.

FCR: The FCR was calculated by dividing the average feed consumption by the average weight gain of the birds in the plots studied.

Mortality was monitored daily to correct feed consumption and conversion considering the weighing of birds and feed on the day of mortality, as described by Sakomura and Rostagno (2016). Viability analysis was calculated using the formula: Viability = 100% - Mortality%.

Meat characteristics, the relative weight of the carcass, and offal

At 42 days of age, at the end of the rearing phase, the birds were fasted for 8 hours and given access to water. Subsequently, they were selected and weighed, and one bird from each plot with a weight within ± 10% of the mean weight of the experimental unit was identified and slaughtered, totaling 30 birds. These birds were rendered insensible by cervical dislocation, and manual bleeding was performed by cutting the jugular vein. After gentle scalding at a temperature of 56°C for 1 minute, the birds were manually plucked.

For the calculation of relative weight, the edible viscera were divided into the liver, gizzard, and heart; and the inedible viscera were divided into the proventriculus, small intestine (duodenum, jejunum, and ileum), ceca, ovary, and oviduct.

All the viscera and abdominal fat were weighed on a semi-analytical balance with a weight of 3200 g. To weigh the gizzard, the food inside the organ was removed while the koilin membrane was maintained. The inedible organs (proventriculus, duodenum, jejunum, ileum, and ceca) underwent slight compression to eliminate the interior contents, and the clean tissue was weighed on a scale with an accuracy of 0.5 grams, according to Valentim *et al*. (2017).

The percentage yield of edible viscera, inedible viscera, and abdominal fat was calculated by the ratio between the average weight of the representative cut of each repetition and the carcass weight, according to the following formula:

$$\textit{Yield of the part X} = \frac{Weight}{Carcass} * 100$$

After identifying each segment of the digestive and reproductive tracts (duodenum, jejunum, jejunum + ileum, ceca, ovary, and oviduct) the segments were sectioned, and then the intestinal contents were emptied. A 90 cm measuring tape (with a precision of 0.1 mm) was used for all segments. To obtain the relative length values, the measurements of each segment were divided by the total length of the organ, and the result was multiplied by 100.

For the assessment of meat characteristics, the breast muscles (pectoralis major) of the slaughtered birds were removed and kept in a cold room for 24 hours at 4 ± 1°C, after which they were taken to the laboratory for analysis. The parameters evaluated were 24 h pH luminosity (L*), red/green content (a*), and yellow/blue content (b*).

The determination of 24 h pH was carried out based on the work of Brossi (2009), using a glass body penetration electrode at four different points of the breast muscle, two in the upper part and two in the lower part. The equipment used was a pH meter (Oakton, pH 300, series 35618) with automatic temperature compensation.

For the analysis of luminosity (L*), red/green content (a*), and yellow/blue content (b*), a portable colorimeter (Minolta Chroma Meter, Model CR-400) was used, and the parameters of the CIElab system were read with a D65 illuminant source calibrated on standard white porcelain with Y=93.7, x=0.3160, and y=0.3323 in the Pectoralis major muscle.

The final value used was the average of three readings obtained at different points of the lesion-free muscle, in the ventral region, two in the cranial region, and two in the central region, with the muscle on an opaque surface. This methodology was based on Brossi *et al*. (2009).

Serum metabolites

Blood was collected by cardiac venipuncture from 1 bird of each experimental repetition, totaling 50 slaughtered birds. For the cardiac puncture, 3 ml syringes with 25 × 0.8mm needles without anticoagulants were used. The birds were placed in dorsal recumbency, and then the needle was inserted along the ventral floor of the thoracic inlet to the heart. The samples were immediately centrifuged for serum separation and then frozen at -20°C until biochemical analysis.

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, and total protein (g/dL) were evaluated. The biochemical tests were analyzed using commercial Cobas 111® kits based on the principles of spectrophotometry. Three readings were performed on a spectrophotometer (Beckman Coulter, DU-800) at a wavelength of 500 nm.

Posture phase performance

At the end of the rearing phase (42 days), the birds received a single feed from day 42 until day 63 of age, still following the distribution used in the previous phase. The diets offered were prepared without the inclusion of the different additives to evaluate the impacts of their supplementation in the rearing phase in the initial laying phase. This single diet was calculated following the recommendations of Rostagno *et al*. (2017), (Table 2).

Table 2 – Percentage and calculated composition of the experimental diet in the initial phase of laying.

Ingredient	Quantity (%)
Corn	54.30
Soybean meal	33.00
Oil	2.50
Limestone	7.40
Dicalcium Phosphate	1.05
Mineral Premix	0.10
Vitamin Premix	0.10
DL. Methionine	0.30
L – Lysine	0.20
Hill	0.10
Salt	0.45
Inert	0.50
Calculated composition	
Metabolizable energy (Kcal/kg)	2800
Crude protein (%)	19.46
Digestible lysine (%)	1.08
Methionine + DigestibleCystine (%)	0.94
Digestible tryptophan (%)	0.23
Digestible threonine (%)	0.68
Calcium (%)	3.07
Available phosphorus (%)	0.3
Sodium (%)	0.16
Crude fiber (%)	2.74

[1]*Vitamin supplement/kg of diet: Folic acid (Min.) 145.4 mg; pantothenic acid (min.) 5,931.6 mg; choline (min.) 121.8 g; niacin (min.) 12.9 g; selenium (min.) 480.0 mg; vitamin A (min.) 5,000,000.0 IU; vitamin B12 (min.) 6,500.0 mcg; vitamin B2 (min.) 2,000.0 mg; vitamin B6 (min.) 250.0 mg; vitamin D3 (min.) 1,850,000.0 IU; vitamin E (min.) 4,500.0 IU; and vitamin K3 (min.) 918.0 mg. 2Mineral supplement/kg: Copper (min.) 7,000.0 mg; iron (min.) 50.0 g; iodine (min.) 1,500.0 mg; manganese (min.) 67.5 g; zinc (min.) 45.6 g*

The daily management consisted of collecting and tallying the eggs (with daily counts of broken, cracked, soft-shelled, and shell-less eggs), providing feed, cleaning egg trays, and taking temperature and humidity readings.

During the initial laying phase (42 to 63 days), 16 hours of light were provided daily throughout the experimental period. This light provision was controlled by an automatic timer, which allows the lights to turn on and off during the night and early morning, following the procedure adopted on commercial farms.

During the laying phase, productive performance was also evaluated. The leftovers of feed from each plot were weighed and subtracted from the amount of feed provided to obtain the feed consumption. For birds that died during the period, their average consumption was subtracted and corrected to obtain the true average consumption for the experimental unit.

The average daily weight gain and total weight gain were evaluated by individually weighing the birds in the experimental plots at the beginning and end of the production cycle. The average egg production was obtained by computing the number of eggs produced, including broken, cracked, and abnormal eggs (soft-shelled and shell-less eggs), expressed as a percentage of the average number of birds in the period (egg/bird/day) and of the number of birds housed at the beginning of the experiment (egg/bird housed).

Egg quality

For the assessment of the external and internal quality of the eggs, three intact eggs were collected from each plot in the morning on the last three days of the experimental period (day 63), totaling 270 eggs. First, the eggs were individually weighed on a semi-analytical scale to obtain the egg weight variable, and then specific gravity analysis was performed.

The specific gravity was determined by immersing the egg in saline solutions of different densities, ranging from 1.065 to 1.125 with a variation of 0.005 between each solution, following the methodology proposed by Castelló *et al*. (1989). The densities were adjusted with a hydrometer, and the eggs were submerged from the lowest to the highest saline concentration.

Afterward, the eggs were cracked, and the albumen, yolk, and shell were manually separated. The yolks were individually weighed on a precision balance. Subsequently, the shells were washed in running water and dried in a natural environment for 72 hours. After the procedure, they were left at room temperature and then individually weighed. The weight of the albumen was calculated as the difference in weight between the whole egg and the yolk plus the weight of the shell.

The height of the yolk and albumen and the diameter of the yolk were measured using a digital caliper and a tripod, with the height of the yolk measured in the central region and the height of the albumen measured approximately 1 cm from the yolk. The yolk index was calculated as the ratio between the height and the diameter of this structure.

The Haugh unit was calculated using a mathematical model following the methodology of Alleoni & Antunes (2001):

$$UH = 100 \log (H + 7{,}57 - 1{,}7W0, 37)$$

where H is the height of the thick albumen (mm) and W is the egg weight (g). (g).

The evaluation of yolk color was performed using a portable colorimeter (Minolta CR 410) to assess the luminosity (L*), red (a*), and yellow (b*) parameters at three different points on the surface of the yolk. The egg color was also assessed using a La Roche colorimetric fan.

Statistical analysis

All data obtained were checked for normality of residuals and homogeneity of variances using the Shapiro-Wilk and Levene tests, respectively. Variables meeting these assumptions were subjected to parametric analysis of variance (ANOVA) using the PROC MIXED procedure. Means were compared using Dunnett's test, where the negative control was tested against other treatments.

For non-parametric variables that did not meet ANOVA assumptions, a logarithmic transformation ($Y' = \log(Y+0.5)$) was applied to stabilize variances and approximate normality. This procedure was justified by the criteria identified in preliminary tests. After transformation, these variables were subjected to the Kruskal-Wallis test with Dunn-Bonferroni *post hoc* correction at a 5% significance level.

Additionally, other treatments were compared to each other using Tukey's test. Finally, data that did not meet statistical assumptions even after transformation were subjected to non-parametric frequency analysis using the chi-square test. All statistical analyses were performed using the SAS 9.3 statistical package (2012), with a significance level of 5%.

RESULTS

Significant differences ($p < 0.05$) were observed for the performance variables of feed conversion from 28 to 35 days of age and from 35 to 42 days of age. Treatment with a commercial antibiotic (Bacitracin) resulted in a reduction in feed conversion compared to the other treatments, as shown in Table 3.

Table 3 – Performance of quails fed diets supplemented with different herbal medicines in the rearing phase.

	Treatments						
Performance	**CN**	**CP**	**FC**	**PC**	**PN**	**EPM**	***p***
21 to 28 days							
MW (g)	92.81	91.08	92.29	92.47	93.79	0.715	0.139
FI (g)	16.97	15.81	16.84	17.02	16.64	0.575	0.576
WG (g)	5.34	5.44	5.73	5.95	5.35	0.212	0.657
FC (g)	3.63	2.92	3.20	3.05	3.40	0.176	0.280
28 to 35 days							
MW (g)	118.05	115.77	118.97	115.79	122.50	2.836	0.451
FI (g)	22.05	21.37	23.85	21.90	22.85	1.120	0.569
WG (g)	4.64	4.92	4.61	4.55	4.70	0.290	0.912
FC (g)	4.58ab	4.25b*	5.65a	4.91ab	4.81ab	0.333	0.006
35 to 42 days							
MW (g)	137.92	139.80	138.17	134.77	144.00	4.472	0.688
FI (g)	4.64	4.90	4.70	4.51	4.76	0.278	0.894
WG (g)	33.06	29.18	32.52	33.57	29.65	1.799	0.297
FC (g)	6.70ab	5.96b	5.98ab	7.61a	6.37ab	0.399	0.037
21 to 42 days							
MW (g)	166.64	153.64	158.24	153.56	165.33	5.211	0.242
FI (g)	24.55	23.87	25.14	24.50	23.71	0.668	0.568
WG (g)	6.02	5.32	6.07	5.93	5.77	0.292	0.389
FC (g)	4.10	4.34	4.17	4.11	4.06	0.193	0.863

*CN: Negative control. CP: Positive control. FC: Commercial herbal medicine. PC: Commercial probiotic. PN: Natural pollen. MW: Middleweight. FI: Feed intake. WG: Weight gain. FC: Feed Conversion. Means followed by different letters on the same line differ from each other according to Tukey's test (p<0.05). EPM: Standard error of the mean. * Means followed by an asterisk on the same line differ from the negative control (NC) according to Dunnett's test (p<0.05).*

No significant differences in organ yield (liver, heart, gizzard, proventriculus, ovary, or oviduct) were detected among the groups receiving different additives ($p>0.05$). The treatments did not affect organ biometry either ($p>0.05$), as shown in Table 4.

No significant difference ($p>0.05$) was detected for serum metabolites (Table 5).

No differences were observed in the meat characteristics of the birds, as shown in Table 6.

No treatment effects were observed on performance in the initial laying phase, as shown in Table 7.

A statistically significant difference ($p<0.05$) was observed in the egg quality variables (Table 8). Albumen height was greater in the group that received the commercial probiotic (BS Gold®), yolk height was greater when the pollen treatment was used, and luminosity was more intense in the control group. The colorimetric fan showed a greater color intensity when pollen was used, and the yolk diameter increased with the use of commercial phytotherapeutics (Phyton 100®).

Table 4 – Biometrics and relative weight of internal organs of quails fed different feed additives in the rearing phase.

	Treatments						
	CN	CP	FC	PC	PN	EPM	p
Organ weight (g)							
Live weight	147.75	141.50	150.50	143.62	145.00	2.908	0.233
Full housing	126.12	125.37	130.79	125.62	125.87	2.561	0.540
Liver	3.09	3.28	3.47	2.97	3.36	0.198	0.395
heart	1.40	1.33	1.36	1.48	1.49	0.075	0.481
Gizzard	4.05	4.15	4.13	4.27	3.58	0.202	0.664
Proventriculus	0.68	0.61	0.67	0.66	0.71	0.037	0.420
Ovary	1.04	1.07	0.62	0.55	0.36	0.415	0.685
Oviduct	2.57	2.89	3.80	1.99	2.92	0.814	0.628
Duodenum	1.44	1.23	1.38	1.18	1.26	0.081	0.158
Jejunum + ileum	2.31	2.02	2.08	2.13	2.16	0.121	0.539
Cecos	1.49	1.26	1.41	1.24	1.35	0.099	0.366
Organ length (cm)							
Intestine	58.81	49.56	52.44	47.50	50.25	1.651	0.161
Duodenum	10.62	9.25	10.28	9.06	9.37	0.393	0.228
Jejunum + ileum	37.93	36.51	38.18	34.06	35.87	1.311	0.187
Cecos	7.66	8.18	8.08	7.12	7.28	0.391	0.241
Oviduct	17.68	20.87	21.98	17.43	16.43	2.669	0.527
Relative percentage of organs (%)							
Liver	3.08	3.25	3.33	2.85	3.24	0.237	0.619
Heart	1.38	1.30	1.28	1.42	1.43	0.054	0.224
Gizzard	3.98	4.07	3.92	4.07	3.71	0.156	0.474
Proventriculus	0.68	0.59	0.64	0.64	0.68	0.044	0.560
Ovary	1.07	1.11	0.60	0.53	0.35	0.442	0.674
Oviduct	18.26	20.77	21.20	16.95	16.01	3.021	0.682

*CN: Negative control. CP: Positive control. FC: Commercial herbal medicine. PC: Commercial probiotic. PN: Natural pollen. EPM: Standard error of the mean. Means followed by different letters on the same line differ from each other according to Tukey's test (p < 0.05). EPM: Standard error of the mean. * Means followed by an asterisk on the same line differ from the negative control (NC) according to Dunnett's test (p < 0.05).*

Table 5 – Serum metabolites of Japanese quails fed different dietary additives in the rearing phase.

	Treatments						
	CN	CP	FC	PC	PN	EPM	p
Serum metabolites							
ALT	1.52	1.83	2.13	1.16	1.59	0.361	0.413
AST	135.0	165.2	172.7	148.3	160.4	21.404	0.749
ALB2	0.64	0.76	0.90	0.72	0.76	0.110	0.591
TP2	1.66	1.83	2.33	1.71	1.75	0.289	0.475

CN: negative control. CP: Positive control. FC: Commercial herbal medicine. PC: Commercial probiotic. PN: Natural pollen. EPM': Standard error of the mean. ALT: alanine aminotransferase. AST: Aspartate aminotransferase. ALB2: Albumin. TP2: Total proteins. Means followed by different letters on the same line differ from each other according to Tukey's test (p < 0.05).

Table 6 – Characteristics of the meat of quails fed different dietary additives during the rearing phase.

	Treatments						
	CN	CP	FC	PC	PN	EPM	p
Characteristics of the meat							
Color L	47.67	47.40	46.82	47.36	47.47	0.853	0.967
Color A	10.79	11.72	11.34	13.03	10.66	0.856	0.316
Color B	4.49	5.97	4.50	5.00	3.98	0.814	0.497
pH 0	5.57	5.61	5.61	5.58	5.76	0.056	0.142
pH 24	5.61	5.66	5.77	5.75	5.75	0.41	0.054

CN: negative control. CP: Positive control. FC: Commercial herbal medicine. PC: Commercial probiotic. PN: Natural pollen. EPM: Standard error of the mean. Means followed by different letters on the same line differ from each other according to Tukey's test (p<0.05). Means followed by an asterisk on the same line differ from the negative control (NC) according to Dunnett's test (p<0.05).

Table 7 – Performance of quails in the initial laying phase fed different dietary additives in the rearing phase.

	Treatments						
Variables	CN	CP	FC	PC	PN	EPM	p
42 to 63 days							
WM (g)	158.20	159.00	156.30	155.44	158.7	0.045	0.156
FI (g)	28.645	29.380	29.99	30.14	30.79	0.18	0.236
WG (g)	1.45	1.67	1.65	1.77	1.69	0.002	0.189
EP (%)	54.73	50.09	53.69	47.44	47.40	3.952	0.562

CN: Negative control. CP: Positive control. FC: Commercial herbal medicine. PC: Commercial probiotic. PN: Natural pollen. WM: Middleweight. FI: Feed intake. WG: Weight gain. EP: Egg production. EPM: Standard error of the mean.

Table 8 – Egg quality of quails fed different dietary additives during the rearing phase.

	Treatments						
Variables	CN	CP	FC	PC	PN	EPM	p
42 to 63 days							
EP	10.58	10.62	10.33	10.51	10.15	0.181	0.344
WE	0.82	0.86	0.83	0.82	0.82	0.016	0.382
PE (%)	7.83	8.15	8.03	7.89	8.17	0.128	0.234
ST	0.22	0.23	0.20	0.53	0.21	0.141	0.429
AH	3.66b	3.85ab	4.14ab	4.16ab	4.33a*	0.137	0.005
AW	6.52	6.62	6.13	6.23	6.24	0.1450	0.081
PA (%)	61.59	62.40	59.33	59.25	61.47	0.8694	0.033
HG	9.42b	9.73ab	9.77ab	9.47ab	9.84a*	0.105	0.011
WY	3.22	3.13	3.37	3.45	3.08	0.104	0.078
GD	22.83a	23.11a	22.88A	23.13a	21.55b*	0.277	0.004
GI	0.41b	0.42b	0.42b	0.41b	0.45a*	0.006	0.001
PY	30.57ab	29.44b	32.63ab	32.85A	30.34b	0.8504	0.018
HU	84.43b	89.25a*	88.55ab	86.47ab	88.38ab	0.8222	0.006
L*	57.07A	55.49ab	56.55ab	56.48ab	54.83b*	0.445	0.003
A	-2.52	-2.21	-2.47	-2.45	-2.30	0.147	0.546
B	40.65	41.48	41.31	40.90	40.14	0.605	0.530
F	4.13ab	3.95b	4.12ab	4.45ab	4.62a	0.167	0.037
SG	67.91	69.37	67.50	66.66	68.12	0.757	0.154

*CN: negative control. CP: Positive control. FC: Commercial herbal medicine. PC: Commercial probiotic. PN: Natural pollen. EPM: Standard error of the mean. EP: Egg weight. WE: Weight of the eggshell. PE (%): percentage of eggshells. ST: Shell thickness. AH: Albumen Height. AW: Albumen weight. PA (%): Percentage of albumen. HG: Height of the gem. WY: Weight of the yolk. GD: Gem diameter. GI: Gem index. PY: Percentage of yolk. HU: Haugh Unit. F: fan. SG: Specific gravity. Means followed by different letters on the same line differ from each other according to Tukey's test (p<0.05). *Means followed by an asterisk on the same line differ from the negative control (NC) according to Dunnett's test (p<0.05).*

DISCUSSION

To optimize animal health and increase production standards, the use of additives in feed as alternatives to antibiotics is increasing, aiming to avoid unwanted residues in meat and eggs as well as microbial resistance resulting from the inappropriate use of antibiotics (Galindo *et al*., 2019; Moraleco *et al*., 2019). Drawing on an ancient practice in cultures such as Ancient Egypt, China, India, and Greece, the use of plant extracts and medicinal plants is gaining prominence. Research indicates that these extracts have antimicrobial properties that control bacterial growth in the intestines, enhancing animal performance by optimizing nutrient digestion and absorption (Galindo *et al*., 2019; Oluwafemi *et al*., 2020).

Phytotherapeutic additives, which act as modulators of the intestinal microbiota and strengthen the immune system in quails, have antimicrobial effects, resulting in improvements in performance and animal health (Souza *et al*., 2018). Although there are few specific studies on the replacement of antimicrobials with phytotherapeutics in quail nutrition, the sustainability and accessibility of these additives suggest their potential to meet the growing demand for quality food (Mostafa *et al*., 2023).

The results of this study differ from previous research findings. Eberhart *et al*. (2021), did not observe significant differences in the performance of Japanese quails when homeopathic sources were introduced into their diets. Similarly, Tenório *et al*. (2017), concluded that the inclusion of zootechnical additives had no impact on quail performance parameters.

The lack of effects on the performance of Japanese quails, even with the addition of zootechnical additives to the diet, is related to the natural resistance of these birds (Barbosa *et al*., 2021). Their adaptability to different climatic conditions and pathogens allows them to maintain high performance in the face of such challenges.

The improvement in feed conversion attributed to the use of commercial antibiotics in feed is related to their ability to select beneficial intestinal microbiota, eliminate harmful microorganisms, and maintain beneficial microorganisms. These antibiotics reduce the growth of harmful microorganisms, controlling their impact on intestinal morphology and consequently increasing nutrient absorption (Cardoso-Junior *et al*., 2021).

Thus, the results of the present study suggest that supplementation with a blend of essential oils, probiotics, and natural pollen can be used in the diet without compromising the yield or biometry of the intestinal or reproductive organs of the birds.

According to Farshid *et al*. (2018), who evaluated the use of thyme and ajowan as alternatives to antibiotics in diets for Japanese quails not exposed to challenges, no significant differences were found in the relative organ weights (liver, heart, and small intestine) in treatments that included these additives compared to the negative treatment without antibiotics.

In this study, the lack of additive supplementation in the control group did not influence the evaluated parameters. The lack of significant results may be associated with the favorable environment in which the experiment was conducted, characterized by good sanitary conditions.

In a study conducted by Naghizadeh *et al*. (2019), Japanese quails were challenged with 200 µLof four different types of *E*. *coli* phages at 25 days of age. The researchers observed a linear reduction in the relative weights of the heart and liver organs on days 2, 3, 5, and 10 after the challenge. Thus, the similarity in the results found in this study may be attributed to the lack of a similar challenge imposed on the quails.

This idea is supported by Valentim *et al*. (2018), who reported that one explanation for the absence of beneficial effects of probiotics in experiments with broiler chickens may be the lack of challenge due to the limited use and good hygiene of the facilities where the experiments were conducted, which do not resemble the sanitary conditions normally found in commercial poultry houses.

ALT and AST are used as indicators of liver damage because their presence in the bloodstream is a sign of cellular changes or damage in the liver. ALB2 and TP2M are indicators of nutritional status and health, reflecting aspects such as liver function, hydration status, and nutrient availability (Diaz Gonzales *et al*., 2018).

Taleb *et al*. (2023), conducted a study on the inclusion of different zootechnical additives in the diet of quails and concluded that there was no significant impact on blood biochemical parameters, corroborating the findings of Eberhart *et al*. (2021) when testing different zootechnical additives.

The blood biochemical metabolites of birds provide indicators of their physiological health, reflecting nutritional influence. When these parameters are within the appropriate standards, they suggest an optimized physiological state for the bird category, indicating that the diets were suitable and did not affect the health of the animals.

Although the results obtained from this research did not show significant outcomes, they did not pose any harm to animal health, indicating that they can be added to the diet without affecting the animals' performance.

The greater luminosity in the eggs from the control group may be associated with the lower deposition of carotenoids in their yolks, as this group did not receive any type of phytotherapeutic supplement in their diet. According to observations by Moreno *et al*. (2016), carotenoids can passively diffuse into enterocytes or be actively absorbed through receptors. The intensity of egg yolk coloration is directly related to the number of carotenoids, whether natural or synthetic.

Therefore, an increase in the coloration of eggs from quail fed diets containing pollen was noted, as evidenced by the colorimetric fan. This increase is associated with the availability of proteins, vitamins, and minerals (K, Ca, and Mg) provided by the addition of pollen to the diet, which enhances the deposition of carotenoids in egg yolks (Maia *et al*., 2020).

Protein may have a beneficial effect on egg quality because it contains antioxidant substances such as flavonoids, carotenoids, and phenolic compounds. Meeting the protein requirements in the diet is essential because it provides essential nutrients for cell differentiation, resulting in trophic alterations that increase the surface area of mucosal absorption in the intestinal tract, thus stimulating greater utilization of dietary nutrients (Karadal *et al*., 2018).

These findings are consistent with the results of this study, which demonstrated an increase in albumen and yolk height in Japanese quails supplemented with pollen in their diet. The greater luminosity in the eggs from the control group may be associated with the lower deposition of carotenoids in their yolks, as this group did not receive any type of phytotherapeutic supplement in their diet. The intensity of egg yolk coloration is directly related to the number of carotenoids present in the diet, whether natural or synthetic, and microbial resistance resulting from the inappropriate use of antibiotics (Moraleco *et al*., 2019). Additionally, the action of microorganisms can affect the availability and deposition of these carotenoids in eggs.

The results suggest that the use of these health-promoting agents in poultry may not only preserve but also improve egg quality, potentially benefiting both production and consumer health. These findings encourage the continued exploration of these natural approaches as sustainable and effective strategies for promoting the quality of poultry products.

The natural resistance of Japanese quails may explain the lack of significant effects of the addition of phytotherapeutics to the diet, while supplementation with pollen has nutritional benefits without adverse effects, indicating the safety of blends of essential oils, probiotics, and natural pollen.

CONCLUSION

A blend of essential oils, probiotics, and natural pollen can replace growth-promoting antibiotics in Japanese quail diets. This substitution did not negatively affect zootechnical performance, serum metabolites, relative weight, or the size of reproductive or digestive organs, and did not affect egg performance or quality in the initial laying phase.

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AUTHOR CONTRIBUTIONS

Conceptualization JZ and JKV; methodology FRC; CMK and RGG software MFCB; validation JZ; JKV and VARCH; formal analysis JZ, AAA and JKV; investigation RGG and MFCB; resources PHB and FCS; data curation FCS and FRC; writing—original draft preparation AAA, DDM and JKV ; writing—review and editing JZ and BBP; visualization BBP; supervision JZ; project administration JZ; funding acquisition JKV. All authors have read and agreed to the published version of the manuscript.

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DATA AVAILABILITY STATEMENT

The data supporting this study will be made available upon request to the authors via email.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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