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Standardized ileal digestible tryptophan requirements for japanese laying quails

Exigência de triptofano digestível para codornas japonesas em fase de postura

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Abstract: This study aimed to estimate the nutritional requirement of standardized ileal digestible (SID) tryptophan for Japanese quails during the initial laying phase and to assess its effects on egg quality, organ weights, leukocyte differential count, and heterophile: lymphocyte ratio. The experimental design was completely randomized, with 5 treatments (0.14%, 0.19%, 0.24%, 0.29%, 0.34%) and 5 replicates, resulting in 25 experimental units with 12 quails per unit, totaling 300 birds aged 42 to 126 days. Based on the results, a SID tryptophan level of 0.14% was optimal for the laying phase. Oviduct weight exhibited a quadratic effect, suggesting a requirement of 0.24% SID tryptophan. A decreasing linear effect was observed in heterophiles and basophils, while lymphocytes and monocytes showed an increasing linear effect. The heterophile: lymphocyte ratio demonstrated a quadratic effect, indicating a requirement of 0.31% SID tryptophan. However, for birds aged 63 to 126 days, 0.14% SID tryptophan was sufficient for optimal performance, with a SID tryptophan: lysine ratio of 13% and a SID tryptophan intake of 34.67 mg per bird per day. Thus, reducing tryptophan levels in the diet did not adversely affect bird performance.

Keywords: egg production; egg quality; essential amino-acids; blood count

Resumo: Este trabalho objetivou estimar a exigência nutricional de triptofano digestível para codornas japonesas na fase inicial de postura, além de verificar seus efeitos sobre a qualidade dos ovos, o peso dos órgãos, a contagem diferencial leucocitária e a relação heterófilo:linfócito. O delineamento experimental adotado foi o inteiramente casualizado, apresentando cinco tratamentos (0,14; 0,19; 0,24; 0,29 e 0,34%) e cinco repetições, resultando em 25 unidades experimentais, com 12 codornas por unidade experimental, totalizando 300 aves no período de 42 a 126 dias de idade. Considerando os resultados, estimou-se o nível de 0,14% de triptofano digestível para a fase de postura. O peso de oviduto apresentou efeito quadrático, possibilitando estimar as exigências de 0,24% de triptofano digestível. Os heterófilos e basófilos apresentaram efeito linear decrescente, os linfócitos e monócitos efeito linear crescente e para a relação heterófilo:linfócito foi observado efeito quadrático, possibilitando

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estimar as exigências de 0,31% de triptofano digestív. No entanto, para esta fase (63 a 126 dias de idade), com base no desempenho, o nível de 0,14% de triptofano digestível foi o mais indicado, relação triptofano:lisina digestível de 13% e consumo de 34,67 mg / ave/ dia de triptofano digestível. Desta forma, a redução do nível de triptofano na dieta convencional não afetará o desempenho das aves.

Palavras-chave: aminoácidos essenciais; perfil hematológico; produção de ovos; qualidade de ovos

1. Introduction

Tryptophan is an essential amino acid with crucial roles in immunity, behavior, food intake, and the synthesis of various metabolites necessary for optimal physiological function in animals. It is involved in complex metabolic pathways that contribute to well-being and performance, and its deficiency can impair vital functions ([1,2]). Quails, being naturally active, may exhibit stressful behaviors that negatively impact egg production. Tryptophan, associated with serotonin synthesis, can reduce agitation and aggression in quails, thus improving egg production ([3]). Additionally, tryptophan promotes increased feed intake, further enhancing egg production ([3]).

As an essential amino acid, tryptophan must be supplemented through diet. It serves as a precursor for niacin and serotonin (5-HT), which is further converted into melatonin. These compounds are involved in mechanisms such as increased feed intake, stress reduction, and various metabolic pathways, including serotonin, melatonin, and nicotinic acid, alongside protein biosynthesis ([2,4]). Adequate levels of tryptophan promote well-being and feed intake, while excessive amounts can lead to satiety ([5]).

Previous studies have provided different recommendations for digestible tryptophan requirements in laying quails. Allen & Young ([6]) suggested 0.17% SID tryptophan for quails in weeks 6 to 12 of laying, while Leeson & Summer ([7]) recommended 0.22% total tryptophan during the production phase. Pinheiro et al. ([8]) advised 0.21% for quails aged 21 to 30 weeks, and Rostagno et al. ([9]) suggested 0.22% to 0.24%, depending on the quail's body weight.

Given the inconsistencies and limited data on essential amino acids such as tryptophan, most research focuses on the first limiting amino acids, so this study aims to enhance the understanding of Japanese quail nutrition during the early laying phase. The study aimed to estimate the SID tryptophan requirement to maximize the zootechnical performance of Japanese quails in the early laying phase (9 to 18 weeks) and to evaluate its influence on parameters affected by tryptophan metabolism.

2. Material and methods

All procedures were by the guidelines of the Animal Experimentation Ethics Committee of the State University of Maringá (Protocol No. 9290010817/2017).

2.1. Animals, facilities, and management

The experiment occurred at the Quail Farming Sector of the Iguatemi Experimental Farm (FEI) from May to September 2016. Japanese laying quails, 42 days old, were used.

These female quails were obtained from a commercial hatchery (Vicami lineage) and were conventionally reared on litter until the experimental period began.

Temperature and relative humidity data were collected daily using thermo-hygrometers placed at two points in the barn (beginning and end), with morning temperatures averaging 20.07°C and 19.11°C, and afternoon temperatures averaging 25.54°C and 22.20°C. Relative humidity ranged from 56.99% to 72.08% in the morning and 63.60% to 50.00% in the afternoon. A natural light program was used during the rearing and growing phases.

At 42 days of age, the quails were transferred to a conventional laying barn, housed in galvanized wire cages (three birds per cage), with nipple drinkers and trough feeders. Water and feed were provided ad libitum. The lighting program began with 14 hours of light (natural + artificial) at 42 days of age, increasing weekly by 30 minutes until reaching 17 hours of light. Light intensity was set to 21 lux/m² using an automatic timer.

2.2. Experimental design and diets

A completely randomized design (CRD) with five treatments and five replications was used. The experimental diets consisted of five levels of digestible tryptophan (0.14%, 0.19%, 0.24%, 0.29%, and 0.34%), with 12 birds per experimental unit, totaling 300 birds. Diets were formulated based on the recommendations and ingredient composition values proposed by Rostagno et al. ([10]), with corn, soybean meal, and corn gluten values determined by near-infrared spectroscopy (NIRS) in a specialized laboratory. The composition of the experimental diets is presented in Table 1.

Table 1. Composition of experimental diets for Japanese quails in the laying phase

	Digestible Tryptophan (%)				
Ingredients	0.14	0.19	0.24	0.29	0.34
Corn (7.87%)	61.094	61.094	61.094	61.094	61.094
Soybean meal (46%)	14.753	14.753	14.753	14.753	14.753
Corn gluten (63%)	6.993	6.904	6.809	6.732	6.655
Glutamic acid	3.000	3.013	3.030	3.030	3.030
Limestone	6.792	6.792	6.792	6.792	6.792
Dicalcium hosphate	1.247	1.247	1.248	1.248	1.248
Salt	0.328	0.328	0.328	0.328	0.328
L-lysine HCl (98.50%)	0.654	0.656	0.657	0.658	0.659
DL-Methionine (99.00%)	0.361	0.364	0.366	0.368	0.370
L-Threonine (99.00%)	0.155	0.157	0.159	0.160	0.162
L-Tryptophan (98.00%)	0.000	0.051	0.102	0.154	0.205
L-Valine (98.50%)	0.150	0.152	0.154	0.156	0.159
Vitamin/mineral premix[1]	0.400	0.400	0.400	0.400	0.400
Inert[2]	4.073	4.091	4.108	4.128	4.146
Total	100.00	100.00	100.00	100.00	100.00

Calculated values					
Metabolizable energy (Kcal/kg)	2.800	2.800	2.800	2.800	2.800
Crude protein (%)	18.800	18.800	18.800	18.800	18.800
Calcium (%)	2.922	2.922	2.922	2.922	2.922
Available phosphorus (%)	0.304	0.304	0.304	0.304	0.304
Sodium (%)	0.146	0.146	0.146	0.146	0.146
Potassium (%)	0.456	0.456	0.456	0.456	0.456
Chlorine (%)	0.354	0.354	0.354	0.354	0.354
Digestible lysine (%)	1.097	1.097	1.097	1.097	1.097
Digestible methionine+cystine (%)	0.900	0.900	0.900	0.900	0.900
Digestible threonine (%)	0.658	0.658	0.658	0.658	0.658
Digestible tryptophan (%)	0.140	0.190	0.240	0.290	0.340
Digestible valine (%)	0.823	0.823	0.823	0.823	0.823
Digestible tryptophan:lysine	12.762	17.320	21.878	26.436	30.994
EBD (mEq/kg^{-1}) [3]	80,40	80,40	80,40	80,40	80,40

[1]Vitamin/mineral premix (guaranteed levels per kg of feed); [2]Fine washed sand; [3]Electrolyte balance in the diet; Trp: tryptophan; Vit.A – 4.500.000 IU; Vit. D3 – 1.250.000 IU; Vit. E – 40 mg; Vit. B1 – 2.78 mg; Vit. B2 – 20 mg; Vit. B6 – 5.25 mg; Vit. B12 – 50 mg; Vit. K3 – 10.07 mg; Calcium Pantothenate – 40 mg; Niacin – 100 mg; Choline – 1400 mg; Zinc – 315 mg; Iron – 245 mg; Manganese – 387.5 mg; Copper – 76.56 mg; Cobalt – 1 mg; Iodine – 4.84 mg; Selenium – 1.27 mg

2.3. Bird performance

Eggs were collected daily at 8:00 a.m. to calculate egg production rate (%) and egg mass (g/egg/bird/day). At the end of each period, quails and feed were weighed to determine average body weight (g), feed intake (g), feed conversion per egg mass (g/g of eggs), and feed conversion per dozen eggs (g/dozen). Mortality was recorded daily to adjust feed consumption and determine the viability of each experimental unit.

2.4. Internal and external quality of quail eggs

The birds were evaluated over four 21-day production cycles, with the last three cycles used for quality analysis. During the final three days of each cycle, internal and external egg quality was assessed, and average egg weight was determined. Specific gravity was measured by immersing all eggs in saline solutions of varying densities, adjusted using a Baumé hydrometer with a precision of 0.005 g/mL (Hamilton, [11]).

For other quality analyses, three eggs with average weight from each experimental unit were selected. The eggs were opened on a glass plate to measure the height (mm) and diameter (mm) of the yolk and albumen using a digital caliper (Digimess, precision of 0.02 mm). Yolk height was measured at its highest point, and albumen height was measured in the region closest to the yolk. The diameter was averaged from two measurements for both the yolk and albumen. These measurements were used to evaluate internal egg quality, determining the yolk index, albumen index, and Haugh Unit (HU). The HU was calculated using the formula: $HU = 100 \log (A + 7.57 - 1.7 \times EW^{0.37})$, where A is albumen height and EW is egg weight.

After opening the eggs, the shells were washed, dried, and stored for later measurement of shell weight and thickness (mm). Thickness was measured at four points around the equatorial region using a micrometer (Mitutoyo, model 700-118 "Quick Mini"). Shell weight per unit area (SWUA) was determined using the formula adapted by Rodrigues et al. ([12]): SWUA = $(SW / 3.9782 \times EW^{0.7056}) \times 100$, where SW is shell weight (g), and EW is egg weight (g).

The yolk and albumen were separated to measure yolk weight, while albumen weight was obtained by subtracting the yolk and shell weights from the total egg weight. The weight data allowed for the calculation of yolk, albumen, and shell percentages by dividing the weight of each component by the total egg weight and multiplying by 100.

2.5. Relative weight of organs

At the end of the four laying cycles, ten birds from each treatment group were euthanized via intravenous administration of the barbiturate thiopental and then sacrificed by cervical dislocation to assess relative internal organ weights. Euthanasia was performed following a six-hour fasting period. After fasting, each quail was individually weighed to determine the live weight at slaughter (LWS). The quails were then eviscerated, and the following organs were weighed: pancreas, proventriculus, liver, intestine, lung, heart, and oviduct. The relative weight (%) of each organ was calculated by dividing the organ's weight (g) by the bird's total weight (g) and multiplying by 100.

2.6. Differential leukocyte count and heterophil: lymphocyte ratio

The differential leukocyte count was conducted at 126 days of age. Blood samples were collected from two birds per experimental unit (totaling 50 samples) via jugular vein puncture using a heparinized syringe. A small portion of each blood sample was used to prepare blood smears on glass slides, which were then stained using the May-Grünwald-Giemsa method. The smears were analyzed under a light microscope (Motic®, ds 300, Xiamen, China) with a 1000x objective.

For each blood smear, 100 leukocytes were examined, including granular types (heterophils, eosinophils, basophils) and non-granular types (lymphocytes, monocytes). The leukocytes were counted using the MsCounter2 application, which allowed for the calculation of the percentage of each leukocyte type. The heterophil-to-lymphocyte ratio was calculated by dividing the percentage of heterophils by the percentage of lymphocytes, following the method described by Campo & Dávila ([13]).

2.7. Statistical analysis

The response variables from the behavioral assessment were quantified as percentages and transformed using the log(x+1) function. Statistical analysis was performed using RStudio statistical software ([14]). To test the effects of the treatments, the model described below was applied, and the assumption of normality of residuals was verified using the Shapiro-Wilk test.

$$Y_{ik} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \varepsilon_{ik}$$

wherein:

Y_{ik} = measured variable in the experimental unit *k* fed a diet containing the i^{-th} level of Trp;

β_0 = general constant;

β_1 = effect of Trp;

β_2 = effect of Trp squared; and

ε_{ik} = random error associated with each observation.

Variables showing treatment effects were analyzed using polynomial regression to determine the best-fitting models (linear or quadratic), based on the optimal data fit for each variable. The quadratic model was used to estimate the nutritional requirements.

3. Results

No significant effect of digestible Trp on the performance and egg quality of Japanese quails during the laying phase (63 to 126 days) was observed (Tables 2 and 3). However, for relative organ weights (Table 4), a quadratic effect was noted for the oviduct weight, with the optimal level estimated at 0.24% digestible Trp.

Table 2. Performance of laying Japanese quails (126 days of age) as a function of digestible tryptophan levels

	Trp (%)					SEM
Variables	0.14	0.19	0.24	0.29	0.34	
BW (g)	169.86	167.80	171.25	170.66	171.65	0.927
FI (g.bird.day^{-1})	24.76	24.68	24.68	23.73	24.60	0.357
LR (%)	86.40	87.19	85.36	83.39	85.20	0.916
EM (g.bird.day^{-1})	9.42	9.48	9.34	9.03	9.33	0.111
VIA (%)	96.67	91.67	95.00	96.67	95.00	1.361
FCR (egg mass)	2.64	2.63	2.66	2.66	2.66	0.032
FCR (dozen eggs)	0.36	0.36	0.36	0.36	0.37	0.004
AFE (days)	55.40	51.80	56.00	54.60	55.00	0.640
DTI (mg.bird.day^{-1})	34.67	46.89	59.23	68.83	83.64	3.579

Trp: digestible tryptophan; SEM: standard error of the mean; BW: body weight; FI daily feed intake; LR: laying rate; EM: egg mass; VIA: viability; FCR (by egg mass): feed conversion ratio by egg mass; FCR (by dozen eggs): feed conversion ratio by dozen eggs; AFE: age at first egg; DTI: digestible tryptophan intake

Table 3. Egg quality of Japanese quails as a function of digestible tryptophan levels

	Trp (%)					SEM
Egg quality	0.14	0.19	0.24	0.29	0.34	
HU	95.61	96.65	96.45	95.58	96.53	0.223
YI	0.50	0.51	0.51	0.52	0.51	0.003
AI	0.16	0.16	0.16	0.15	0.16	0.002
SG ($g.mL^{-1}$)	1.07	1.07	1.07	1.07	1.07	0.000
EW (g)	10.89	10.87	10.93	10.82	10.97	0.048
SW (g)	0.84	0.83	0.84	0.83	0.83	0.006
YW (g)	3.29	3.34	3.34	3.29	3.30	0.021
AW (g)	6.75	6.70	6.75	6.69	6.84	0.034
% Shell	7.71	7.65	7.65	7.69	7.57	0.036
% Yolk	30.22	30.68	30.53	30.41	30.12	0.142
% Albumen	62.07	61.67	61.81	61.90	62.32	0.149
SWSA	3.92	3.88	3.89	3.90	3.85	0.020
ST (mm)	0.20	0.21	0.21	0.20	0.21	0.001

Trp: digestible tryptophan; SEM: standard error of the mean; HU: Haugh Unit; YI: yolk index; AI: albumen index; SG: specific gravity; EW: egg weight; SW: shell weight; YW: yolk weight; AW: albumen weight; SWSA: shell weight per surface area; ST: shell thickness

Table 4. Relative organ weight of laying Japanese quails (126 days of age) as a function of digestible tryptophan levels

	Trp (%)					SEM
Organs	0.14	0.19	0.24	0.29	0.34	
Pancreas (%)	0.25	0.25	0.26	0.22	0.23	0.012
Proventriculus (%)	0.52	0.48	0.53	0.49	0.45	0.011
Intestine (%)	4.25	4.20	4.22	4.04	4.17	0.120
IL (cm)	63.23	65.30	65.37	60.15	62.23	1.043
Liver (%)	3.72	3.25	3.40	3.31	3.41	0.108
Lung (%)	0.73	0.75	0.74	0.77	0.77	0.020
Heart (%)	0.87	0.83	0.77	0.84	0.85	0.017
Oviduct (%)	5.05	6.82	7.75	5.87	5.56	0.360
Regression equations			P-value	R^2	Estimates	
			Trp		Trp (%)	
$Ov= -4{,}312+95{,}862Trp-199{,}429Trp^2$			<0,019(Q)	0,90	0,24	

Trp: digestible tryptophan; SEM: standard error of the mean; IL: intestinal length; cm: centimeters; Ov: oviduct; R^2: coefficient of determination; Q: quadratic effect

In the differential leukocyte count (Table 5), digestible Trp did not affect eosinophils and heterophils. Basophils showed a decreasing linear trend, while lymphocytes and monocytes exhibited an increasing linear trend. The heterophil-to-lymphocyte ratio displayed a quadratic effect, with the optimal level estimated at 0.31% digestible Trp.

Table 5. Differential leukocyte count and heterophil:lymphocyte ratio in laying Japanese quails (126 days of age) as a function of digestible tryptophan levels

	Trp (%)					SEM
Blood cells	0.14	0.19	0.24	0.29	0.34	
Heterophil (%)	52.00	35.60	33.20	32.20	21.40	2.032
Lymphocyte (%)	35.40	52.60	55.40	55.80	68.80	2.216
Monocyte (%)	3.80	3.80	4.20	5.60	5.40	0.337
Eosinophil (%)	5.20	5.40	5.60	5.00	3.20	0.353
Basophil (%)	3.60	2.60	1.60	1.40	1.20	0.341
H:L	1.48	0.68	0.60	0.58	0.31	0.082
Regression equations			P-value	R^2	Estimates	
			Trp		Trp (%)	
H= 65,888–129,200Trp			<0,001 (L)	0,84	-	
L= 20,000+140,000Trp			<0,001 (L)	0,82		
M= 2,160+10,000Trp			<0,033 (L)	0,95	-	
B= 3,452-6,800Trp			<0,009 (L)	0,90	-	
H:L=3,599-20,381Trp+32,286Trp^2			<0,001 (Q)	0,84	0,31	

Trp: digestible tryptophan; SEM: standard error of the mean; H: heterophil; L: lymphocyte; M: monocyte; B: basophil; H:L: heterophil:lymphocyte ratio; R^2: coefficient of determination; L: linear effect; Q: quadratic effect

4. Discussion

Tryptophan is believed to stimulate feed intake through several mechanisms. Higher tryptophan levels significantly increased ghrelin, suggesting enhanced ghrelin expression in the gastric fundus and plasma, thus stimulating appetite, as ghrelin is a key hormone for appetite regulation ([15]). Landeiro & Quarantine ([16]) report that tryptophan administration can double serotonin synthesis, though further evidence is needed to confirm whether increased serotonin release significantly enhances feed intake.

In this study, no significant effects of digestible Trp levels on performance variables were observed. Therefore, the lowest level used (0.14%) is recommended for laying Japanese quails, as it showed minimal variation in the analyzed variables. Increasing tryptophan levels did not contribute to increased feed intake or performance maximization. The dietary tryptophan levels might have been insufficient to observe significant effects. This finding aligns with Rizzo et al. ([17]), who found no influence of tryptophan levels on the performance of Japanese quails in lay, with the lowest level (0.23% Trp) being sufficient.

The NRC ([18]) recommends 0.19% digestible tryptophan for laying Japanese quails, a value higher than the one recommended in this study. Rostagno et al. ([9]) determined a requirement of 0.24% digestible tryptophan, which may be higher due to differences in quail body weight. Studies on amino acid requirements in laying Japanese quails suggest a requirement of 0.17% Trp, which closely aligns with the 0.14% digestible Trp recommended in this study ([4]). Other studies recommend slightly higher levels of tryptophan, depending on the phase of laying ([19, 20]).

Another critical factor is determining the tryptophan ratio to ensure effective conversion into its metabolic products (serotonin, melatonin, niacin), while also supporting cellular renewal and maintaining an ideal nutritional balance ([21]). Based on the results, diets for laying Japanese quails (63 to 126 days of age) can be formulated with 0.14% digestible Trp and an ideal tryptophan ratio of 13%, with a daily intake of 34.67 mg. Proper determination of requirements during the laying phase is crucial for maintaining adequate egg production and prolonging the laying period ([22]).

The likely lower digestible tryptophan requirement in this period may be due to well-balanced diets that meet all nutritional needs, showing that minimal supplementation of tryptophan is sufficient for proper development without affecting laying performance ([17, 22]). Regarding egg quality, all analyzed variables showed similar values regardless of digestible tryptophan levels, indicating that this amino acid does not significantly influence key characteristics like egg weight, shell quality, yolk, and albumen.

Santos et al. ([23]) reported that albumen constitutes 60% of an egg's mass, primarily made up of water and proteins, notably albumin. Yolk represents 30-32% of an egg's weight, consisting of water, proteins, and lipids. Our findings are consistent with these data, indicating that tryptophan does not significantly affect these nutritional and quality attributes.

The relative weight of reproductive organs, another variable assessed, appeared to be influenced by tryptophan levels. Specifically, the weight of the oviduct exhibited a quadratic response, increasing up to a dietary inclusion of 0.24% digestible tryptophan before declining. This pattern fits well with the physiological demands of laying quails, though higher levels of tryptophan were found to impede oviduct development without affecting egg production or egg weight ([24]). This suggests a potential regulatory role of tryptophan in the development of the oviduct in laying Japanese quails ([25]). According to Lima ([26]), higher tryptophan levels enhance the growth of the magnum and uterus, suggesting that tryptophan supplementation could foster oviduct development.

In birds, alterations in the hematological profile can be indicative of stress from environmental, nutritional, or pathological stressors ([27]). A commonly used indicator of avian stress or well-being is the heterophil-to-lymphocyte ratio, which shifts in response to increased heterophils and decreased lymphocytes ([28]). When performing differential leukocyte count analyses, it is crucial to acknowledge that results can vary due to multiple factors such as species, population, genetic background, territory, habitat, sex, age, physiological state, season, management practices, stocking density, and the number of samples analyzed, among others ([29]).

At 126 days of age, our study observed a decrease in the percentage of heterophils and an increase in lymphocytes with higher levels of digestible tryptophan, suggesting a stress reduction. The presence of lower tryptophan levels appeared to correlate with higher stress levels among the birds, indicated by increased heterophils. Rosa et al. ([28]) noted that an elevation in heterophils typically signals stress or infection in animals.

This pattern may be linked to decreased availability of tryptophan for serotonin synthesis, which is crucial for regulating stress-related behaviors. Sarcinelli et al. ([30]) suggested that tryptophan can diminish aggressive behaviors in Japanese quails. Therefore, supplementing diets with higher levels of tryptophan during the laying period could serve as a strategy to alleviate stress in these birds.

The heterophil-to-lymphocyte (H) ratio proves to be a reliable measure of well-being in chickens, with increases in heterophils and decreases in lymphocytes reflecting diminished well-being ([28]). In this study, the H ratio declined as digestible tryptophan levels increased, supporting its use as an indicator of social stress as per Gross & Siegel ([31]), who defined stress levels in chickens using this ratio: 0.2 for low stress, 0.5 for intermediate, and 0.8 for high stress.

From our findings, the highest tryptophan level (0.34%) correlated with low stress, whereas intermediate levels (0.19%, 0.24%, and 0.29%) corresponded to moderate stress, and the lowest level (0.14%) indicated high stress. This confirms tryptophan's role in enhancing well-being and immune regulation in Japanese quails ([30,31]). Moreover, the stress level at the lowest tryptophan concentration surpassed the thresholds set by Gross & Siegel ([31]), indicating severe stress. Given that quails are inherently lively and prone to stress, this behavior could have heightened the observed stress levels, or perhaps the mechanisms that regulate stress were less effective at this minimal tryptophan level.

During inflammatory responses, the enzyme indoleamine 2,3-dioxygenase (IDO) is activated, which metabolizes tryptophan into compounds that modulate the immune system by either inhibiting T lymphocyte proliferation or inducing apoptosis of these cells ([32]). In stressful situations, cortisol production increases, which stimulates the expression of tryptophan 2,3-dioxygenase (TDO). Both IDO and TDO play significant immunoregulatory roles in the tryptophan depletion mechanism ([1, 33]).

Our findings indicate that higher dietary levels of tryptophan led to an increased number of lymphocytes, suggesting a state of well-being potentially linked to the serotonin pathway. Serotonin, a neurotransmitter, influences behavior, stress hormone secretion, and immune function ([34]). The hematological values determined for laying Japanese quails in this study are critical for setting reference standards for this species, addressing the existing gaps in the literature. Maintaining well-being during the laying period is crucial for reducing stress and boosting immune function in quails, leading to healthier birds, and potentially maximizing egg production.

5. Conclusion

In conclusion, a dietary requirement of 0.14% digestible tryptophan, corresponding to a digestible tryptophan ratio of 13% and a daily intake of 34.67 mg/hen/day, was adequate to sustain performance and egg quality in Japanese quails during the laying phase (63 to 126 days of age). However, the observation of significant differences in some variables with varying levels of digestible tryptophan suggests that tryptophan also plays a crucial role in the development of reproductive organs and stress reduction

Conflict of Interest Statement

The authors declare that there is no conflict of interest.

Author Contributions

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