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(Original Article)

Assessment of Aloe Vera Gel as a Feed Additive on Productive Performance, Reproductive Traits, and Intestine Histology in Japanese Quail (*Coturnix Coturnix japonica*).

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Abstract

Two experiments were designated to study the effect of Aloe Vera gel as a natural feed additive on productive, reproductive performance, and intestine histology of Japanese quail during growth and laying periods. In the growing period, 240 unsexed chicks were randomly assigned to four treatment groups, 4 replicates each (15 birds/ replicate). The treatments were the control group fed basal diet while T1, T2, and T3 were fed the basal diet supplemented with 0.25, 0.50 and 1.0g Aloe Vera gel /kg diet, respectively. In the laying period, 144 birds were distributed into 4 treatments and 4 replicates each. The treatments were the control group fed basal diet, while T1, T2, and T3 were fed the basal diet supplemented with 0.75, 1.5 and 3.0g Aloe Vera gel /kg diet, respectively.

Results showed positive response to Aloe Vera gel in females only ($P \leq 0.05$) during the growing period. Blood parameters in both sexes were within normal physiological levels. The results of duodenum histology in both male and female showed significant increase ($P \leq 0.05$) in villi heights and VH/CD ratios in all treatments over the control group. Results of the second experiment revealed no treatments effect on egg weight, fertility or hatchability %. Steroid assay indicated that levels of testosterone in males or estrogen in females were decreased ($P \leq 0.05$) by the treatments. In conclusion, Aloe vera gel enhanced growth performance but caution should be exercised with higher doses due to potential negative impacts on reproductive function.

Keywords: *Aloe Vera gel, Egg production, Growth, Intestine Histology, Quail.*

Introduction

Japanese quails (*Coturnix coturnix* japonica) are counted extremely good economic value in agriculture sector with efficient productive ability, secure and safe quality, which meet consumer demand and satisfaction. In fact, females lay 250 - 300 eggs annually, starting at age 6-week-old. The incubation period for quail eggs is short and takes around 17 days. Lately, these birds have become widely used for egg and meat

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production. Quails can be reared on the ground or in cages requires small space (Vargas-Sánchez *et al.,* 2019; Lukanov *et al.,* 2023).

The global industry of these birds is still unregulated and hasn't official published statistics. However, 1.4 billion quails are raised annually for meat and eggs. More than 80% of the worldwide quails are found in China. Actually, the European Union produces more than 100 million quails (Kokoszyński *et al*., 2024).

In Egypt, quail is considered a cheap source for meat production categorized by high-quality protein, low-caloric content, and deliciousness. Quail meat has gained much popularity among consumers and become ideal food for all consumer ages (Mahrose *et al.,* 2022). The frequent application and misuse of drugs as growth promoters in poultry feed have led to rise of antimicrobial resistance that adversely obstructed poultry industry. Medicinal plants such as Aloe vera were adopted to achieve the targeted nutritional and health status due to their modest availability and low cost. Unlike synthetic antimicrobial, these plants leave no residues in the animal tissue reflecting their safety to consumers. They improve the performance of body functions, reduce susceptibility to infectious diseases, and improve growth and feed efficiency (Islam *et al.,* 2017).

Aloe Vera gel, the mucilaginous substance found in the inner part of the Aloe Vera leaf, possesses a range of biological activities such as antioxidants, antimicrobial, immunomodulatory, and anti-inflammatory effects. These properties may contribute to improved gut health, enhanced nutrient absorption, and better growth performance in poultry. Moreover, Aloe Vera has been reported to positively affect reproductive functions, potentially through its phytoestrogenic components and its role in improving metabolic status. (Qiao *et al.,* 2013; Muscogiuri*, et al.,* 2020). Accordingly, the study aims to assess the impact of Aloe Vera gel as a natural feed additive on growth performance, reproductive parameters, and intestinal histology in Japanese quail. It is hypothesized that Aloe Vera gel inclusion in the diet could enhance performance traits and gut health, providing a sustainable alternative to conventional growth promoters.

Materials and Methods

This study was carried out at the Research unit of the Poultry Production Department, Assiut University, during the period from 28 January 2021 to 6 May 2021.

Experiment 1. Effects of Aloe Vera gel on growth performance of Japanese quails

1. Birds, housing, diets, experimental design, and managerial procedures

Two hundred and forty 7-day-old unsexed Japanese quail chicks were used in this experiment over the growing period (7-40 day-old). Chicks were individually wing-banded, weighed, and randomly assigned to four dietary treatments. Each treatment (60 birds) was further sub-divided into four replicates (15 chicks/R). Chicks were kept in a galvanized battery with pen dimensions of (75*50*45cm). Treatments were as follows: group 1; received basal diet without any supplementation and kept as a control (T1). The 2nd, 3rd, and 4th Groups received the same basal diet supplemented with 0.25, 0.5, and 1.0 g Aloe vera gel /kg diet, respectively. Following NRC (1994) instructions, the basal corn-soybean meal diet was formulated to satisfy all nutritional needs of the growing

Japanese quail (Table 1). Clean fresh water was available 24 hrs. The experimental birds received similar managerial, environmental, and health conditions, in accordance with animal welfare guidelines.

2. Data collection and parameters measured

- Growth performance

The weekly live body weight (LBW) of chicks was recorded using electronic digital balance to the nearest 0.1 g. Additionally, the weekly feed consumption (FC) of each replicate was calculated by subtracting the amount of feed left at the end of the week from the amount of feed supplied at the beginning of the same week. Weekly body weight gain (BWG) and feed conversion ratio (FCR) were calculated using the formulas:

$$BWG = \frac{\text{BW } week\ n\ - \text{BW } week\ n-1}{7 \text{ days}}$$

$$\text{FCR } = \frac{\text{FC}}{\text{BWG}}$$

Table 1. Composition and calculated analysis of the experimental diets for growing and laying Japanese quail

Ingredients	Diet composition %	
	Grower	Layer
yellow Corn	54.20	58.0
Soybean 44%	40.0	31.5
Corn oil	2.0	1.50
Limestone	1.0	5.50
L-lysine	0.10	0.10
DL/methionine	0.10	0.15
Di-calcium phosphate	2.0	2.65
Premix[1]	0.30	0.30
Sodium chloride	0.30	0.30
Total %	100.00	100.00
Calculated analyses		
M E (k cal/kg)	2970	2847
Crude protein %	22.9	19.5
Crude fiber %	4.1	3.1
Crude fat (=EE) %	4.38	3.96
Calcium %	0.88	2.60
Available phosphorus %	0.44	0.44
Methionine %	0.52	0.47
Lysine %	1.18	1.13
Arginine %	1.40	1.12

[1] Each Kg of Vitamin minerals premix contains *vit. A:* 130,000 IU. D3: 26,000 IU; vit. E: 120 IU; vit B12: 150 µg; vit. K_3 MSB: 16 mg; vit B2: 50 mg; capantothenate B3 5: 120 mg; nicotinic acid PP: 250 mg; Thiamine B1: 25 mg; folic acid: 15 mg; pyridoxine B6: 15 mg; Betain-Choline-HCl: 5000 mg; Mn: 700 mg; Zn: 600 mg; Fe: 400 mg; Cu: 40 mg; Iodine: 7 mg; Co: 2 mg; Se: 1.5 mg; B.H.T.: 1250 mg; Zinc bacitracin: 150 mg.

- Internal organs

At 40 days of age, 12 birds from each group (3 birds/ replicate) representing the ABW of the replicate ±10% were selected to ascertain some carcass characteristics. Birds were slaughtered and the weights of their internal organs (bursa, spleen, ovary, and tests) were recorded and expressed as percentage of LBW.

- Blood analysis

Two vials of blood samples were collected from each bird. The first sample was collected in an EDTA vial to estimate the packed cells volume (PCV%) and White blood cell differential (Hawkey and Dennett, 1989). The second vial was collected in plain tube without anticoagulant and centrifuged at (3000 rpm/15 minutes) for serum separation. The separated serum was collected in Eppendorf tubes and preserved in a freezer (-20 C) for later analysis; liver enzymes ALT (Alanine Aminotransferase) and AST (Aspartate Aminotransferase) by spectrophotometer instrument using a commercial kit (BIOMED). Also, glucose and cholesterol concentrations were estimated in the blood serum using the Egyptian company for (S.A.E) spectrum purpose of estimation.

- Histological examination of the duodenum

Following slaughter, the duodenal digesta were removed using normal saline. Then, three 1-cm-long segments from the upper, middle, and bottom portions of each bird's duodenum were fixed in 10% buffered formalin. After complete fixation, the duodenum samples were washed with tap water, dehydrated in ascending serials of ethanol up to absolute alcohol, cleared in xylol, and embedded in paraffin. A microtome Leica RM 2235 (Leica Biosystems Nussloch GmbH, Germany) was used to cut sections with 5µm thickness from each segment. Sections were stained with hematoxylin and eosin. The histological methods were performed according to Romeis, (1989). A lighting microscope with a digital camera was used to take photos of stained sections. Villi height and width and crypt depth were measured by using Image J software.

Experiment 2. Effects of Aloe vera gel on laying quails and their reproductive performance

This experiment was carried out to evaluate the effect of dietary supplementation of Aloe Vera gel on reproductive performance of quails' laying hens for a period (6 - 14 weeks of age).

1. Birds, housing, diets, experimental design, and managerial procedures

A total of 144 birds from experiment 1 were assigned to 4 treatments (36 birds) and further divided into 4 replicates each (9 quails/each, including 6 females and 3 males). The birds received basal diet that meet all nutrient requirement according to the NRC (1994) as shown in Table 1. The treatments were as follows: group1 basal diet and served as a control group (T1), then groups 2,3 and 4 received the basal diet supplemented with Aloe Vera gel at concentrations 0.75, 1.5, and 3.0 g/kg, respectively. Feed and fresh water were available *ad libitum*. Birds were raised under similar managerial, environmental, and health conditions, in accordance with animal welfare guidelines.

2. Data collection and parameters measured

- Feed consumption

Daily and total feed consumption (g) during the experimental period in each group were recorded according to the following equation:

$$\text{Average individual daily FC (g/bird/week)} = \frac{\text{Supplied feed, g} - \text{Remaining feed, g}}{\text{Number of birds} \times \text{Number of days}}$$

Average individual weekly FC = Σ FC

- Egg mass

Eggs were collected daily, weighed using electronic digital balance to the nearest 0.1 g, and the average egg production was determined per hen (Ghanem *et al*., 2017).

$$\text{Average egg mass (egg / hen/ day, g)} = \text{The laying percentage} \times \text{the average daily egg weight, g}$$

- Laying rate

After optimum sexual maturity (25 % of egg production= at 9 weeks old), all eggs were daily collected from each group. The average egg production per bird on a "hen day basis" was calculated. A hen day average was attained by dividing the number of eggs laid during a given period by the average number of birds on hand during the same period (Alodan and Mashaly, 1999).

$$\text{Egg production \% during the month} = \frac{\text{Total no. of eggs laid}}{\text{Actual no. of hen days}} \times 100$$

$$\text{Percent of egg production during the month} = \frac{\text{Total eggs laid in the month}}{\text{Actual number of Hen days}} \times 100$$

- Feed conversion rate (FCR)

The amount of feed the birds needed to produce kg eggs (kg feed/kg eggs) was calculated from weekly egg mass production and weekly feed consumption over the experimental period to verify the feed conversion of each group according to Clark et al. (2019) following the equation;

$$\text{FCR (per kg Egg mass)} = \frac{\text{Weekly feed consumption}}{\text{Weekly egg mass output}}$$

- Egg Fertility and Hatchability

The daily collected eggs over 8 weeks were marked and refrigerated (4 ± 1°C) for up to 5 days. The eggs were mechanically incubated for 18 days at controlled humidity and temperature (60% and 37.8°C, respectively). The egg turning stopped at the first 3 days of incubation. The hatching process begins on the 17th day and ends on the 23rd day. The number of eggs containing dead embryos, pecked eggs, cracked eggs, loose eggs, and hatched chicks were counted.

Finally, the fertility % of the eggs was calculated for each group using the following equation:

$$\text{Fertility (\%)} = \frac{\text{Total fertile eggs}}{\text{Total eggs set}} \times 100$$

Hatchability results were calculated (Ghanem *et al*., 2017) by the equation;

$$\text{Hatchability on fertile egg basis (\%)} = \frac{\text{No. of chicks hatched}}{\text{Total fertile eggs}} \times 100$$

- Reproductive organs traits

- Gonads

At the end of the trial period (age 14 weeks), 3 females and 3 males from each treatment (total of 24 birds), were weighed then slaughtered and disemboweled. The gonads (female ovary and oviducts and the male tests) were surgically dissected, weighed, and measured using calipers. The percentage of ovarian weight and the right and left testicle were calculated. After weighing the female reproductive organs, the mature follicles counted and weighed.

- immune organs

Bursa of Fabricius, and spleen were surgically dissected, weighed, and measured using calipers.

- Serum Testosterone and Estrogen levels

At slaughter time, blood samples were collected from birds of each group in a blood collecting tube without anticoagulant. Serum was separated, transferred carefully to clean dry vials and frozen (-20 ºC) until analysis for assaying the testosterone (T) and estrogen (E) levels using specific kits (ZEUS Diagnostic, Inc. New Jersy, USA and Enzyme immunoassay Test Kit, Catalog Number 10000, Parkin Elmer Health Science, Inc. USA, respectively) according to manufacturer's instructions.

- Total Anti-oxidant Capacity (TAC)

The serum level of total antioxidants (TAC) was calorimetrically determined using a commercial kit (Total Antioxidant Capacity Assay Kit ab65329, Abcam, Cambridge, UK), following the manufacturer's instructions.

3-Statistical analysis

All variables measured in this study were subjected to a one-way analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of SAS software (version 9.3, 2012). Duncan's multiple range test (Duncan, 1955) was used to determine the significant differences among all treatment means.

$$Y_{ik} = \mu + T_i + e_{ik},$$

Where: Y is the dependent variables; μ is the general mean; T is the treatment effect, and e is the random error.

Duncan's multiple range test (1955) with a 5% level of probability was used to determine the difference among means when the treatment effects were significant.

Results and Discussion

- **Growth performance**

- Body weight (BW) and body weight gain (BWG), feed consumption (FC) and feed conversion ratio (FCR)

The effect of dietary supplementation of Aloe Vera gel on BW and BWG daily FC and FCR of growing female and male Japanese quails from 7 to 40 day- old period is presented in Table 2. In female quails, dietary inclusion of 1.0g Aloe Vera gel /kg diet (T3) compared to the control and treatments received lower levels of Aloe Vera gel T1 and T2 resulted in a significant improvement in final body weight ($P \leq 0.01$) compared to the control and groups receiving lower inclusion levels (T1 and T2). The final BW of T3 birds was 228.59 ± 1.54 g, compared to 222.97 ± 0.17 g (control), 221.27 ± 1.77 g (T1), and 221.44 ± 0.96 g (T2). A similar trend was observed for BWG, where T3 birds showed the highest BWG ($P \leq 0.01$). Interestingly, FC was significantly reduced ($P \leq 0.01$) in the T1 group compared to both the control and T3, but it was not statistically different from the T2 group. No significant differences were observed among the treatments for FCR.

Table 2. Effect of dietary supplementation of different levels of Aloe Vera gel on (IBW, FBW, FBWG, FC and FCR[1]) of growing females and males' Japanese quails (Coturnix Coturnix japonica) (X± SE)

Sex	Variables	Control	Aloe Vera gel (g/kg diet)			Prob.
			T1	T2	T3	
			0.25	0.50	1.0	
Female	IBW	40.11±0.15	40.58±0.43	40.05±0.58	39.65±0.66	NS
	FBW	222.97^{b}±0.17	221.27^{b}±1.77	221.44^{b}±0.96	228.59^{a}±1.54	**
	FBWG	182.86^{b}±0.09	180.69^{b}±0.52	181.39^{b}±0.19	188.94^{a}±0.50	*
	F.C	24.08^{a}±0.05	23.34^{b}±0.24	23.75^{ab}±0.24	24.12^{a}±0.24	*
	FCR	3.81±0.03	3.79±0.01	3.81±0.02	3.74±0.04	NS
Male	IBW	39.95±0.62	38.38±0.22	39.01±0.50	39.45±0.60	NS
	FBW	221.85^{a}±0.79	210.17^{b}±2.11	220.10^{a}±1.75	218.21^{a}±1.24	**
	FBWG	181.9^{a}±0.35	171.79^{b}±0.56	181.09^{a}±0.32	178.76^{a}±0.07	**
	F.C	24.13^{a}±0.07	23.24^{b}±0.08	24.20^{a}±0.11	24.12^{a}±0.22	**
	FCR	3.80±0.04	3.89±0.04	3.81±0.03	3.88±0.03	NS

$^{a, b, c}$ Means within the same row with different superscripts are significantly different ($P \leq 0.05$). NS= non-Significant

[1] IBW=initial body weight, FBW= final body weight, FBWG= final body weight gain, FC=feed consumption/ day, FCR= feed conversion ratio

Regarding growth performance parameters of male's quails (Table 2), no differences were found in the final BW among treatments T3, T2 and the control, quite the opposite, the lowest final BW (210.17±2.11g) was recorded for males fed on the lowest level of Aloe Vera gel, 0.25 g/kg diet. Likewise, final BWG followed same trend of BW results ($p \leq 0.05$). Additionally, T1 recorded the lowest BWG ($P \leq 0.01$) of all treatments (171.79±0.56g). The reduction in BW and BWG can be elucidated by the lower FC during the experimental trial in group T1. Subsequently, FCR verified no difference among treatments. The observed improvement in BW and BWG with Aloe Vera supplementation may be related to its bioactive components, such as polysaccharides, vitamins, minerals, and amino acids, which are known to enhance

digestion, nutrient absorption, and overall health (Muscogiuri *et al*., 2020). Consistent with our findings, previous studies in poultry have reported reduced FC and improved growth performance with Aloe Vera gel supplementation (Sinurat *et al*., 2002; Darabighan *et al*., 2011; Olupona *et al*., 2010; Hassanbeigy-Lakeh *et al*., 2012; Darabighan and Nahashon.,2014; Nghonjuyi *et al*., 2015; Tariq *et al*., 2015; Shokraneh *et al*., 2016; Nalge *et al*., 2017, and Jogeswar *et al*., 2022). Also, our results of improvement in BW and BWG are consistent with recent studies in quails by Hafeez *et al*. (2024).

- **Internal organs**

The effects of dietary supplementation of Aloe Vera gel on some internal organs of females and males' Japanese quails are presented in Table 3. In female quails, a significant difference ($P \leq 0.05$) was observed in ovary weight among the treatment groups. The highest ovary weight was recorded in T2 birds (0.34 ± 0.01 g), while the lowest was found in T3 birds (0.13 ± 0.01 g). Intermediate values were observed in T1 (0.22 ± 0.02 g) and the control group (0.27 ± 0.02 g). These results suggest that a moderate level of Aloe Vera gel (0.5 g/kg diet; T2) may support reproductive organ development, whereas a higher dose (1.0 g/kg; T3) may suppress ovarian growth. With respect to the bursa of Fabricius, birds in the T3 group recorded the highest bursa weight (0.18 ± 0.01 g; $P \leq 0.05$), followed by T2 (0.14 ± 0.02 g). No significant differences were observed between T1 (0.09 ± 0.02 g) and the control group (0.08± 0.01 g), indicating that lower levels of Aloe Vera gel (0.25 g/kg) exerted minimal effect on bursal development.

The increase in bursa weight observed in the T3 group may suggest a potential immunostimulatory effect of Aloe Vera at this inclusion level. The bursa of Fabricius is a key lymphoid organ in birds responsible for B-cell maturation, and its development is closely linked to immune competence. Aloe Vera contains biologically active compounds such as acemannan, a polysaccharide known for its immunomodulatory and antimicrobial properties, which may contribute to enhanced immune organ development. These findings support the role of Aloe Vera as a natural immunostimulant in poultry, capable of influencing internal organ development, particularly at optimized dietary levels (Darabighane and Nahashon, 2014). Also, our findings are coinsided with those reported by Jiang *et al.,* (2006), Feng *et al.,* (2011); Akhtar *et al.,* (2012) Darabighane *et al*., (2012) and Besharatian *et al*., (2012). Spleen weight showed no significant differences among the treatment groups, indicating that Aloe Vera gel supplementation had no measurable effect on spleen development during the study period. However, LBW at slaughter was significantly higher ($P \leq 0.05$) in birds from T3 (217.00 ± 1.53 g) and T2 (215.33 ± 2.33 g) compared to the control group (208.00 ± 0.58 g) and T1 (201.67 ± 1.45 g). These results reflect the positive influence of moderate to high levels of Aloe Vera gel supplementation on overall body mass at market age. Regarding male internal organ weights (testes, bursa, and spleen), the results presented in Table 3 showed no significant differences among the treatment groups. This suggests that male quails were either less responsive to Aloe Vera gel supplementation compared to females, or that the levels used in this trial were below the threshold needed to elicit

a physiological effect on these organs. Similarly, LBW at slaughter did not differ significantly among treatments. The recorded values were 193.00 ± 0.01 g (control), 193.67 ± 2.19 g (T1), 196.00 ± 1.00 g (T2), and 195.00 ± 0.58 g (T3). These findings further indicate that Aloe Vera gel supplementation, at the tested levels, had limited influence on carcass weight in male quails during the experimental period.

Table 3: Effect of dietary supplementation of different levels of Aloe Vera gel on some internal organs of growing females and males' Japanese quails (Coturnix Coturnix japonica) (X± SE)

Sex	Traits	Control	Aloe Vera gel (g/kg diet)			Prob.
			T1	T2	T3	
			0.25	0.50	1.0	
Female	LBW(g)	208.00^{b}±0.58	201.67^{c}±1.45	215.33^{a}±2.33	217.00^{a}±1.53	**
	OVR(g)	0.27^{b}±0.02	0.22^{c}±0.02	0.34^{a}±0.01	0.13^{d}±0.01	**
	SPL(g)	0.05±0.02	0.07±0.01	0.07±0.01	0.07±0.01	NS
	BUR(g)	0.08^{c}±0.01	0.09^{c}±0.02	0.14^{b}±0.02	0.18^{a}±0.00	**
Male	LBW(g)	193.00±0.00	193.67±2.19	196.00±1.00	195.00±0.58	NS
	TES(g)	1.98±0.31	2.17±0.10	2.66±0.42	2.52±0.44	NS
	SPL(g)	0.02±0.01	0.03±0.01	0.02±0.00	0.04±0.01	NS
	BUR(g)	0.10±0.01	0.10±0.01	0.09±0.02	0.12±0.01	NS

LBW: live body weight (g), OVR: ovary(g), SPL: spleen(g), BUR: bursa(g), TES: tests(g)
^{abc} Means within the same row with mutual superscript are not significantly different (P < 0.05). NS: Non-Significant

- **Hematological parameters, differential count of leukocytes and liver enzymes**

The outcomes of hematological parameters and liver enzyme activities are presented in Tables 4 and 5. In female quails, serum glucose and cholesterol levels (Table 4) were significantly increased (P ≤ 0.05) in all Aloe Vera-supplemented groups (T1, T2, and T3) compared to the control. Conversely, packed cell volume (PCV%) values were significantly lower (P ≤ 0.05) in all treated groups (T1, T2, and T3) relative to the control group. Despite these differences, all recorded hematological values remained within normal physiological ranges, suggesting that Aloe Vera gel supplementation did not induce any pathological alterations in blood parameters. This supports the conclusion that the tested levels of Aloe Vera were not harmful to female quails.

Regarding liver enzyme activities, both aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were significantly elevated (P ≤ 0.05) in the treatment groups compared to the control. However, the enzyme levels still fell within normal physiological concentrations, indicating no evidence of hepatotoxicity. This finding is consistent with Tariq *et al*., (2015), who also reported no toxic effects of Aloe Vera gel on liver function when administered at appropriate dietary levels (Tariq *et al*., 2015).

The results of hematological and biochemical parameters in male quails are presented in Table 4. A significant increase in blood glucose levels (P ≤ 0.05) was observed in the T1 and T3 groups compared to the control and T2. Additionally, cholesterol levels were significantly elevated (P ≤ 0.05) in all Aloe Vera-supplemented groups T1, T2, and T3 compared to the control. The recorded values were 242.90 ± 0.23 mg/dL (T1), 268.16 ± 1.63 mg/dL (T2), and 280.47 ± 4.63 mg/dL (T3), in contrast to

192.15 ± 1.53 mg/dL in the control group. These findings are in agreement with previous studies by Egger *et al*., (1996) and Jagmohan *et al*., (2013), which reported that dietary Aloe Vera gel could stimulate increases in blood glucose levels in mice due to presence of acemannan in Aloe Vera. Similarly, AST and ALT enzyme levels were significantly higher ($P \leq 0.05$) in all Aloe vera-treated groups compared to the control. Despite this elevation, enzyme values remained within physiologically acceptable ranges, suggesting no hepatotoxic effects. These results are consistent with the findings of Arif *et al*., (2022), who concluded that Aloe Vera supplementation did not produce any harmful effects on liver function or blood biochemistry in poultry.

Table 4. Effect of dietary supplementation of different levels of Aloe Vera gel on blood parameters and liver enzymes of growing females and males' Japanese quails (*Coturnix Coturnix japonica*) (X± SE)

Sex	Traits	Aloe Vera gel (g/kg diet)				Prob.
		Control	0.25	0.50	1.0	
		Blood parameters				
Female	**Glucose**	138.67^{b}±0.72	167.70^{a}±4.83	170.26^{a}±3.02	163.26^{a}±2.65	**
	Cholesterol	138.65^{d}±1.30	146.49^{c}±0.98	203.27^{a}±1.63	182.86^{b}±1.27	**
	PCV	45.00^{a}±0.29	35.33^{d}±0.67	40.00^{b}±0.29	37.50^{c}±0.00	**
		Liver enzymes				
	AST	26.85^{b}±0.64b	30.94^{b}±2.59	37.74^{a}±0.50	42.52^{a}±0.78	**
	ALT	6.06^{b}±0.19	6.71^{b}±0.74	6.80^{b}±0.44	9.26^{a}±0.05	**
		Blood parameters				
Male	**Glucose**	162.83^{b}±0.33	186.17^{a}±0.37	162.72^{b}±0.83	163.26^{a}±2.65	**
	Cholesterol	192.15^{d}±1.53	242.90^{c}±0.23	268.16^{b}±1.63	280.47^{a}±4.63	**
	PCV	42.50±1.44	41.17±0.73	39.17±0.17	40.67±0.33	**NS**
		Liver enzymes				
	AST	23.87^{ab}±1.61	27.99^{ab}±1.42	29.58^{b}±3.09	32.99^{a}±1. 92	*
	ALT	5.13^{c}±0.25	9.01^{b}±0.71	9.65^{b}±0.17	11.94^{a}±0.71	**

GLU: Glucose (mg/dl), CHOL: Cholesterol (mg/dl), PCV: Packed Cell Volume (%)
a, b, c Means within the same row with different superscripts are significantly different ($P \leq 0.05$). NS= non-Significant
ALT: Alanine Aminotransferase (IU/L). AST: Aspartate Aminotransferase (IU/L).

As shown in Table 5, the lymphocyte percentages in female quails were not significantly different among the treatment groups. Heterophil percentages were also similar across treatments, with the exception of T2, which showed a significantly lower heterophil count ($P \leq 0.05$) compared to the control, T1, and T3 groups.

Monocyte percentages were elevated in T1 and T2 compared to both the control and T3, indicating a possible immunomodulatory response at moderate Aloe Vera supplementation levels. Additionally, eosinophil counts were significantly increased in T2 compared to the control and other treatment groups. No significant differences were observed in basophil counts among all treatments. The lowest heterophil percentage (25.33 ± 1.33%) observed in T2 was accompanied by the lowest heterophil-to-lymphocyte (H/L) ratio (0.48 ± 0.03), which may suggest reduced physiological stress and better immune stability in this group. The H/L ratio is a well-established indicator of stress response in birds, and lower values are generally associated with improved welfare and immune function.

As shown in Table 5, the lymphocyte percentage in male quails was significantly higher in groups T1 (55.00 ± 0.01%) and T3 (53.67 ± 1.86%) compared to the control (49.67 ± 0.33%) and T2 (47.33 ± 0.33%) ($P \leq 0.05$). This suggests a potential immunomodulatory effect of Aloe Vera at lower and higher inclusion levels. Eosinophil counts showed no significant differences among treatments, indicating a stable eosinophil response regardless of Aloe Vera gel supplementation. The results for basophils and the heterophil-to-lymphocyte (H/L) ratio displayed an inconsistent trend, with no clear pattern across treatment groups. These findings are in agreement with previous studies by Valle Paraso *et al.,* (2005), Altug *et al*., (2010), Darabighane *et al*., (2011), Mahdavi *et al.,* (2012), Singh *et al.,* (2013), Al-Qaysi and Taha (2015), and Yadav *et al*., (2017), who reported that Aloe Vera supplementation positively influenced white blood cell profiles, particularly lymphocyte and heterophil counts, in male birds. The immunostimulatory effect of Aloe Vera is largely attributed to its bioactive compounds, such as acemannan, known for enhancing immune cell proliferation and activity.

Table 5. Effect of dietary supplementation of different levels of Aloe Vera gel on deferential WBC of growing Japanese quails (*Coturnix Coturnix japonica)* (X± SE)

Sex	Traits	Control	Aloe Vera gel (g/kg diet)			Prob.
			0.25	0.50	1.0	
Female	Lymphocyte %	50.00±0.00	49.33±0.33	55.33±4.06	47.67±1.45	NS
	Heterophils%	35.00^{a}±0.00	34.33^{a}±0.33	25.33^{b}±1.33	36.67^{a}±0.67	**
	Monocyte %	10.00^{b}±0.00	11.67^{a}±0.33	12.33^{a}±0.33	10.67^{b}±0.33	**
	Eosinophil %	4.00^{b}±0.00	4.33^{b}±0.33	6.33^{a}±0.67	4.33^{b}±0.33	*
	basophils %	1.00±0.00	0.33±0.33	0.67±0.33	0.67±0.33	NS
	H/L	0.70^{a}±0.00	0.70^{a}±0.01	0.48^{b}±0.03	0.77^{a}±0.04	**
Male	Lymphocyte %	49.67^{b}±0.33	55.00^{a}±0.00	47.33^{b}±0.33	53.67^{a}±1.86	**
	Heterophils%	34.67^{ab}±0.33	25.00^{c}±0.00	35.67^{a}±0.88	28.00^{bc}±3.51	*
	Monocyte %	11.00^{b}±0.00	14.50^{a}±0.29	11.00^{b}±0.58	12.67^{ab}±1.20	*
	Eosinophil %	4.00±0.00	5.50±0.29	5.00±0.00	5.33±0.88	NS
	basophils %	0.67^{ab}±0.33	0.00^{b}±0.00	1.00^{a}±0.00	0.33^{ab}±0.33	**
	H/L	0.70^{ab}±0.01	0.45^{c}±0.00	0.75^{a}±0.02	0.53^{bc}±0.09	*

$^{a, b, c}$ Means within the same row with different superscripts are significantly different ($P \leq 0.05$). NS= non-significant
Hph: Heterophil (%), Lph: Lymphocytes (%), Mph: Monocyte (%), Eph: Eosinophil (%), Bph: basophils (%), H/L: Heterophil ratio

- **Histological examination of the intestinal morphometrics**

The results of duodenal histometric measurements are presented in Table 6. Significant differences ($P \leq 0.05$) were observed in villus height (VH) among all dietary treatments in both female and male quails. In females, villus height increased progressively with higher levels of Aloe Vera gel supplementation: (T1: 892.66 ± 15.97, T2: 951.32 ± 6.68, T3: 957.47 ± 8.88, compared to the control: 792.75 ± 17.55 µm). A similar trend was observed in males: T1: 890.94 ± 12.69, T2: 931.45 ± 9.72, T3: 1069.54 ± 11.84, compared to the control: 877.84 ± 10.73 µm.). In contrast to villus height, crypt depth (CD) values in females showed a significant decrease ($P \leq 0.05$) with increasing levels of Aloe Vera gel, while no significant differences were observed in males. Consequently, the villus height to crypt depth (VH:CD) ratio increased significantly ($P \leq 0.05$) in both sexes. In females, the VH:CD ratios were:

(T1: 9.30 ± 0.32, T2: 10.80 ± 0.44, T3: 11.28 ± 0.11, compared to control: 7.53 ± 0.57). While, in males: (T1: 10.09 ± 0.86, T2: 10.29 ± 0.27, T3: 11.83 ± 0.16, compared to control: 9.56 ± 0.47).

These findings suggest that dietary Aloe Vera gel supplementation improved intestinal histology, as evidenced by increased villus height and VH:CD ratio, particularly at higher inclusion levels. Improved duodenal structure may enhance nutrient absorption capacity, contributing to the observed improvements in growth performance. This aligns with previous studies by Moghaddam and Alizadeh-Ghamsari (2013), Darabighane *et al*., (2011), Kadhim *et al*., (2012), Rodjan *et al*., (2018), Zayed *et al*., (2020), and Hafeez *et al*., (2024), who also reported that Aloe vera positively influences intestinal development in poultry.

Table 6. Effect of dietary supplementation of different concentrations of Aloe Vera gel on the intestinal histometric of growing Japanese quails (*Coturnix Coturnix japonica*) (X± SE)

Sex	Aloe Vera gel (g/kg diet)					Prob
	Traits	Control	0.25	0.50	1.0	
Female	VH	792.75^{c}±17.55	892.66^{b}±15.97	951.32^{a}±6.68	957.47^{a}±8.88	**
	CD	105.70^{a}±3.70	98.35^{ab}±5.58	89.83^{cb}±2.94	84.93^{c}±2.44	*
	VH:CD	7.53^{c}±0.57	9.30^{b}±0.32	10.80^{a}±0.44	11.28^{a}±0.11	**
Male	VH	877.84^{c}±10.73	890.94^{cb}±12.69	931.45^{b}±9.72	1069.54^{a}±11.84	**
	CD	94.88±1.93	91.08±1.53	90.92±2.00	90.46±0.78	NS
	VH:CD	9.56^{b}±0.47	10.09^{ab}±0.86	10.29^{ab}±0.27	11.83^{a}±0.16	**

[a, b, c] Means within the same row with different superscripts are significantly different (P ≤ 0.05). NS= non-significant
VH: Villus Hight(µm), CD: Crypt depth(µm), VH:CD; Villus Hight to Crypt depth ratio(µm)

2. Reproductive performance

- Feed consumption (FC), feed conversion rate (FCR) and Mortality%

The results presented in Table 7 indicate that dietary supplementation with Aloe Vera gel had no significant effect on feed consumption (FC) or feed conversion ratio (FCR) during the reproductive phase. These findings suggest that Aloe Vera gel, at the inclusion levels tested, did not influence feed efficiency in reproductive quails.

These results are consistent with earlier studies by Sultan (2003), Durrani *et al*., (2006), and Moorthy and Edwin (2006), who also reported that supplementation of 0.1% Aloe Vera powder in the diets of White Leghorn layers did not affect feed intake or feed conversion efficiency.

Additionally, mortality rates remained low and were not significantly different among treatments, indicating that Aloe Vera gel supplementation did not negatively affect bird health or survivability during the reproductive period.

- Egg weight (EW), egg mass (EM), egg number (EN) and egg laying rate (ELR)

The results in Table 7 show that egg weight (EW) was not significantly affected by dietary Aloe Vera gel supplementation across all treatment groups. This indicates that Aloe Vera gel inclusion, regardless of the level, did not influence the size or mass of individual eggs. However, significant differences (P ≤ 0.05) were observed in egg laying rate (ELR), egg mass (EM), and egg number (EN). Birds fed the highest level of Aloe

Vera gel (3.0 g/kg diet) exhibited a reduction in all three parameters compared to the control and lower supplementation levels. In contrast, quails fed lower Aloe vera gel levels (0.75 and 1.5 g/kg diet) maintained performance values comparable to the control, with no adverse effects on egg production metrics. These findings suggest that while moderate Aloe Vera gel supplementation may support reproductive performance, higher levels may negatively affect laying efficiency, possibly due to metabolic or hormonal disturbances. Our results are in agreement with those reported by Hasan (2014) and Sarabi and Torshizi (2015), who found that Aloe Vera leaf gel supplementation in the drinking water of quails had no significant effect on egg weight, supporting the observed neutrality of Aloe Vera on EW in this study.

Table 7. Effect of dietary supplementation of different concentrations of Aloe Vera gel on feed consumption (FC) g/bird/day, feed conversion ratio (FCR)g feed/g egg, and Egg production (EP) of laying hens of Japanese quails (X± SE)

Traits	Control	Aloe Vera gel (g/kg diet)			Prob<
		0.75	1.5	3.0	
FC	33.53±0.66	33.55±0.86	33.86±1.14	34.78±0.84	**NS**
FCR	2.69±0.04	2.89±0.13	2.80±0.19	2.91±0.13	**NS**
EW	12.55±0.13	11.83±0.31	12.46±0.35	12.28±0.32	**NS**
ELR	54.09^{a}±0.51	54.61^{a}±0.52	53.79^{a}±2.36	45.16^{b}±2.11	**
EM	5.84^{a}±0.06	6.08^{a}±0.16	6.17^{a}±0.67	4.64^{b}±0.49	**
EN	22.72^{a}±0.21	22.94^{a}±0.22	22.59^{a}±0.99	19.69^{b}±0.81	**

$^{a, b, c}$ Means within the same row with different superscripts are significantly different (P ≤ 0.05). NS = non-significant
EN: Egg Number (e/h/m), EW: Egg Weight (g), EM: Egg Mass (g/h/day), ELR: Egg laying rat (%), FC: Feed consumption (g/h/day), FCR: Feed conversion rate (g feed/g egg).

- sexual and internal organs characteristics

According to the results presented in Table 8, there were no significant differences between the control and Aloe Vera-supplemented groups in terms of live body weight (LBW) at slaughter, ovary weight (OVA), and spleen weight (SPL) in female quails. However, the relative weight of the bursa of Fabricius was significantly reduced (P ≤ 0.05) in groups receiving 0.75 and 1.5 g Aloe Vera gel /kg diet compared to the control (0.01 ± 0.00 vs. 0.02 ± 0.01, respectively), indicating a possible immunosuppressive effect at these inclusion levels.

Results showed that male LBW at slaughter was significantly decreased in the T1 group (0.75 g/kg Aloe Vera gel) compared to the control, but did not differ significantly from T2 (1.5 g/kg) or T3 (3.0 g/kg) groups. Interestingly, the T1 group also exhibited a significant increase in spleen percentage (P ≤ 0.05) compared to all other treatments and the control, suggesting a possible immune response at this supplementation level.

Furthermore, the bursa percentage was significantly higher (P ≤ 0.05) in the T2 group compared to the control (0.05 ± 0.00 vs. 0.04 ± 0.00, respectively), whereas the T3 group showed the lowest bursa weight among all treatments (0.03%), indicating a possible suppressive effect at the highest Aloe Vera concentration. Data also revealed that testes weights decreased in males fed higher Aloe Vera concentrations (T2 and T3). This finding aligns with Asgharzade *et al*., (2015), who reported that dietary inclusion of 150 or 300 mg/kg Aloe Vera reduced testicular weight in broilers.

Table 8. Effect of dietary supplementation of different levels of Aloe Vera gel on carcass traits, ovary and testis weights of Japanese quail (*Coturnix Coturnix japonica)* at 2 months (X± SE)

Sex	Traits	Control	Aloe Vera gel (g/kg diet)			Prob
			0.75	1.5	3.0	
Female	LBW(g)	266.00±13.32	298.33±1.20	283.67±4.91	286.00±4.73	NS
	OVA	4.00±0.00	3.67±0.33	3.33±0.33	3.67±0.33	NS
	SPL(g)	0.05±0.01	0.04±0.00	0.04±0.00	0.02±0.00	NS
	BUR(g)	0.02^{a}±0.01	0.01^{b}±0.00	0.01^{b}±0.00	0.02^{a}±0.00	*
Male	LBW(g)	247.33a±8.09	224.33^{b}±2.40	240.00^{ab}±9.24	229.33^{ab}±3.33	**
	SPL(g)	0.04^{b}±0.00	0.05^{a}±0.00	0.03^{b}±0.01	0.04^{b}±0.00	*
	BUR(g)	0.04^{b}±0.00	0.04^{b}±0.00	0.05^{a}±0.00	0.03^{c}±0.00	**
	Tests(g)	3.31^{a}±0.16	3.34^{a}±0.23	2.49^{b}±0.23	2.85^{b}±0.12	**

LBW: Living body weight (g), ova (%): ovary, SPL: spleen, BUR: bursa, Tests: Right testis (g).
^{abc} Means within the same row with mutual superscript are not significantly different (P ≤ 0.05). NS: Non-Significant

- Egg fertility and hatchability

The effects of dietary Aloe Vera gel supplementation on egg fertility and hatchability percentages are presented in Table 9. The results showed that Aloe Vera supplementation at different levels had no significant effect on either fertility or hatchability across all treatment groups. These findings contrast with those reported by Jegde (2011), who observed that high dietary levels of Aloe Vera may act as reproductive inhibitors, leading to reduced egg fertility and histological damage to the gonads of both males and females, thereby potentially impairing reproductive performance. Our results suggest that, within the tested dosage range, Aloe Vera gel does not negatively impact reproductive success in Japanese quails.

Table 9. Effect of dietary supplementation of different concentrations of Aloe Vera gel on fertility and hatchability of laying Japanese quails (X± SE)

Traits	Control	Aloe Vera gel (g/kg diet)			Prob.
		0.75	1.5	3.0	
Fertility%	91.71±2.78	84.35±0.71	83.02±6.87	89.59±2.85	NS
Hatchability%	54.10±2.31	52.92±0.77	51.33±6.96	58.38±0.85	NS

NS: Non –significant

- Serum Testosterone (T), Estrogen (E) levels and Total antioxidant capacity (TAC)

The effects of dietary Aloe Vera gel supplementation on serum testosterone levels in males and estrogen levels in females are presented in Table 10. The results showed that dietary inclusion of Aloe Vera gel at 1.5% (T2) and 3.0% (T3) significantly reduced testosterone levels in male quails compared to the control group (0.14 ± 0.02 and 0.31 ± 0.01 vs. 0.57 ± 0.09 ng/mL, respectively). In contrast, the lowest supplementation level (0.75 g/kg, T1) maintained testosterone concentrations close to the control, indicating minimal disruption at this dosage. A similar pattern was observed for estrogen concentrations in females, where T2 and T3 groups showed significantly lower estrogen levels (48.42 ± 0.83 and 47.32 ± 1.88 pg/mL, respectively) compared to the control (75.66 ± 0.71 pg/mL). These hormonal changes correlate with the previously mentioned reductions in testis and ovary weights observed at higher Aloe Vera gel levels (Table 8),

suggesting a dose-dependent suppressive effect on reproductive organ development and function.

Our findings align with those reported by Mourupoju and Sundaresan (2018) and Jiwantare and Dhurvey (2022), who found that Aloe Vera gel supplementation (25 mg/kg) reduced testosterone levels in male albino rats. Additionally, Selvage *et al*., (2004) demonstrated that phytohormones derived from Aloe Vera hydrogel can exert a hypothalamic suppressive effect, reduce gonadotropin-releasing hormone (GnRH) synthesis and promote hypothalamic-pituitary-gonadal axis degeneration.

The effects of dietary supplementation of Aloe Vera gel on total antioxidants Capacity in male Japanese quails are presented in Table 10.

The results indicated that adding Aloe Vera gel at different levels, exhibited that there were no significant differences between the different treatments groups for total antioxidants level. Hence, more research is needed to study higher levels of Aloe Vera gel on antioxidant supplementation. Our results agreed with those reported by Ibe *et al*., (2013), indicated that Aloe Vera has a low antioxidant content.

Table 10: Effect of dietary supplementation of different levels of Aloe Vera gel on the sexual hormones (T and E) and Total anti-oxidants (TAC) of Japanese quails (X±SE)

Traits	Control	Aloe Vera gel (g/kg diet)			Prob.
		0.75	1.5	3.0	
Testosterone, T (ng/ mL)					
Male	0.57^{a}±0.09	0.55^{a}±0.10	0.14^{c}±0.02	0.31^{b}±0.01	**
Estrogen, E (pg/mL)					
Female	75.66^{a}±0.71	73.64^{a}±0.95	48.42^{b}±0.83	47.32^{b}±1.88	**
TAC, mmol/100 g					
Female	36.65±1.45	33.55±0.05	35.95±0.85	35.30±1.10	NS

^{abc} Means within the same row with mutual superscript are not significantly different (P ≤ 0.05). NS: Non-Significant
TAC= total anti-oxidant capacity

Conclusion

In conclusion, Aloe vera gel shows promise as a natural feed additive to enhance growth performance and intestinal health in Japanese quails when used at appropriate levels, but caution should be exercised with higher doses due to potential negative impacts on reproductive function.

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تقييم تأثير جل الصبار كمضاف غذائي على الأداء الإنتاجي، والصفات التناسلية، ونسيج الأمعاء في طائر السمان اليابان (Coturnix Coturnix japonica)

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الملخص

اجريت تجربتين لدراسة تأثير جل نبات الصبار كمضاف غذائي طبيعي على الأداء الإنتاجي والتناسلي والتشريح النسيجي لأمعاء السمان الياباني خلال فترة النمو وفترة وضع البيض. في فترة النمو، تم توزيع الكتاكيت غير المجنسة عشوائيًا على أربع معاملات، 4 مكررات (15 طائرًا / مكرره). كانت المعاملات كالاتي: المجموعة الضابطة التي غذيت على العليقة الأساسية بينما تم تغذية T1 وT2، T3 على العليقة الأساسية مضافًا إليها 0.25 ،0.50، 1.0 جم من جل نبات الصبار / كجم من العليقة، على التوالي. في فترة وضع البيض، تم توزيع الطيور على 4 معاملات، 4 مكررات (9 طيور لكل منها). كانت المعاملات: المجموعة الضابطة التي غذيت على العليقة الأساسية، بينما تم تغذية T1، T2، T3 على العليقة الأساسية مضافًا إليها 0.75 ،1.5، 3.0 جم من جل نبات الصبار / كجم من العليقة، على التوالي. وقد أظهرت النتائج استجابة ايجابيه للألوفيرا وكانت واضحة لدى الإناث ($P \leq 0.05$) أكثر من الذكور خلال فترة النمو. أظهرت قياسات الدم مستويات مرتفعة ($P \leq 0.05$) من الجلوكوز والكوليسترول وAST وALT في كلا الجنسين، ولكن القيم كانت ضمن المستويات الطبيعية. أظهرت نتائج قياس الخملات للاثني عشر في كل من الذكور والإناث زيادة كبيرة ($P \leq 0.05$) في ارتفاع الخملات ونسبة VH / CD في جميع المعاملات مقارنة بالمجموعة الضابطة. اظهرت نتائج التجربة الثانية عدم وجود تأثير للمعاملات على وزن البيض والخصوبة ونسبة الفقس. اوضحت تركيزات الهرمونات الاستروديه إلى أن مستويات هرمون التستوستيرون لدى الذكور أو هرمون الاستروجين لدى الإناث قد انخفضت ($P \leq 0.05$) ببين المعاملات. يمكن استنتاج ان، جل الصبار يعمل على تعزيز أداء النمو، ولكن يجب توخي الحذر مع الجرعات العالية بسبب التأثيرات السلبية المحتملة على الوظيفة التناسلية.

الكلمات المفتاحية: *السمان، إنتاج البيض، هلام الألوفيرا، هستولوجى الأمعاء، النمو.*