

1 **Effect of Dietary Supplementation of Giloy (*Tinospora cordifolia*) and Ascorbic Acid**  
2 **along with Different Bedding Materials on Egg shell thickness and egg shell weight of**  
3 **Japanese quail**  
4  
5

6 **ABSTRACT**

7 The study investigated the effects of giloy (*Tinospora cordifolia*) and ascorbic acid  
8 supplementation along with different bedding materials (sand B<sub>1</sub>, saw dust B<sub>2</sub> and wheat  
9 straw B<sub>3</sub>) on egg production status of Japanese quails. A total of 432 chicks (7 day-old)  
10 Japanese quails were divided into three equal groups (144 each) for different bedding  
11 material used and each group was further subdivided into four groups (each of 36 chicks) on  
12 the basis of dietary treatment (control T<sub>0</sub>, giloy T<sub>1</sub>, ascorbic acid T<sub>2</sub> and combination of both  
13 T<sub>3</sub>). Thus birds were randomly and uniformly distributed in a total of 12 treatment groups  
14 comprising of 36 birds in each group and each group was further divided into two replicates  
15 comprising of 18 birds in each. Quails were fed with a basal diet (control, T<sub>0</sub>) and the basal  
16 diet supplemented with giloy 5 g/kg of diet, ascorbic acid 240 mg/kg diet and a combination  
17 of giloy 5 g/kg and ascorbic acid 240 mg/kg diet in dietary treatment groups T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>,  
18 respectively. Highly significant (p<0.01) effect of incorporation of supplements and different  
19 bedding material was found on egg shell thickness and egg shell weight of Japanese quail. In  
20 present experiment, egg shell thickness and egg shell weight were higher in saw dust bedding  
21 material group. The interaction between dietary supplementation and different bedding  
22 materials was found non-significant on these traits.

23 **Key words:** Ascorbic acid, Giloy, Japanese quail, egg shell thickness, egg shell weight

24 **INTRODUCTION**

25 Poultry is one of the fastest growing components of the agricultural sector in India.  
26 Poultry plays an important role as animal protein source in human diet in terms of egg and  
27 meat. India ranks fourth in total production of poultry meat in the world (Basic Animal  
28 Husbandry Statistics, 2019). Japanese quail (*Coturnix coturnix japonica*) is one of the most  
29 efficient biological machines for converting feed into animal protein of high biological value  
30 (Das *et al.*, 2012). They have less feeding requirement (about 20-25 g per day) compared to  
31 chicken (120-130 g per day) (Ani *et al.*, 2009).

32 Various types of feed additives, such as antibiotics, enzymes, hormones, prebiotics,  
33 probiotics, herbal products *etc.*, are being used as growth stimulants in poultry production to  
34 improve efficiency and get maximum returns in the shortest possible time. *Tinospora*

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35 *cordifolia*, which is known by the common names guduchi, giloy and heart-leaved  
36 moonseed, it is a herbaceous vine of the family Menispermaceae indigenous to tropical  
37 regions of the Indian subcontinent (Sengupta *et al.*, 2011). Giloy is a rich source of protein  
38 and micronutrients, such as iron, zinc, copper, calcium, phosphorus, and manganese (Saeed *et*  
39 *al.*, 2020). The most clearly established functional role for vitamin C involves collagen  
40 biosynthesis. Beneficial effects result from ascorbic acid in the synthesis of “repair” collagen  
41 (Bera *et al.*, 2010).

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42 Wood sawdust is the most common used bedding material, however there are many  
43 alternative materials that may be used such as peanut hulls, rice and wheat straw, rice hull ash  
44 (Chamblee and Yeatman, 2003), and other dry, absorbent and low-cost organic materials.  
45 Moreover, sand is occasionally used as a bedding material (Shields *et al.*, 2005). Birds spend  
46 their entire life in contact with the litter material. Therefore, its quality is considered a crucial  
47 factor of poultry welfare. Hence, the present experiment was planned to study the effect of  
48 bedding materials such as sand, wheat straw and saw-dust with dietary supplementation of  
49 giloy herb (*Tinospora cordifolia*) and ascorbic acid and their combination on egg quality  
50 traits such as egg shell thickness, egg shell weight in Japanese quail.

## 51 MATERIALS AND METHODS

52 The present study was conducted at Poultry unit, Livestock Farm Complex, College  
53 of Veterinary and Animal Science, Bikaner, Rajasthan University of Veterinary and Animal  
54 Sciences, Bikaner (India) following approval of Institutional Animal Ethics Committee of the  
55 College.

### 56 Experimental Design and Material used

57 The study was undertaken on four hundred and thirty two (432) seven-day old  
58 Japanese quail chicks which were purchased from central poultry development organization,  
59 Chandigarh. Out of 432 birds 72 birds were slaughtered at the age of 8 weeks for evaluating  
60 carcass characteristics and the remaining 360 birds were further used for the remaining traits  
61 (growth traits and egg production traits) for the entire experimental trail of 24 weeks.

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62 The factorial design (3x4) was adopted for the present study. The chicks were equally  
63 and randomly divided into four dietary treatment groups (n=108) and one-third of each  
64 (n=36) were reared on each of the three bedding materials (sand, saw-dust and wheat-straw)  
65 using two replicates each of 18 birds (R1-R2) to make sure ensure the uniformity in various  
66 treatment groups. The chicks were reared on sand, saw-dust and wheat straw as group B<sub>1</sub>, B<sub>2</sub>

67 and B<sub>3</sub>, respectively (n=144 each), and each bedding group was subdivided equally in one  
68 control and three dietary treatment groups (giloy @ 5 g/kg diet, ascorbic acid @ 240 mg/kg  
69 diet, and a combination of both at the said rates) denoted by T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>, respectively  
70 (n=36 each) to study the effect of bedding material, dietary treatment and their interaction on  
71 egg production of birds.

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72 Commercially available readymade starter and finisher and layer rations were  
73 procured as basal diets and feed additives such as giloy (*Tinospora cordifolia*) and ascorbic  
74 acid were supplemented in them. During the first seven days, newspapers were spread on  
75 the litter material and from 8<sup>th</sup> day onward till the completion of this experiment, chicks were  
76 reared on respective litter material of about 6 inches of depth.

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#### 77 **Egg shell thickness (mm)**

78 Egg shell was dried in the air, thereafter drying shell thickness was measured by using  
79 screw gage gauge and three measurements were taken for each egg shell, one on large end, one  
80 on small end and the other one on the equator region. The mean of all three measurements  
81 was considered as thickness of egg shell.

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#### 82 **Egg shell weight**

83 The egg shell along with the membrane was sunk in 5 % EDTA solution for 30  
84 minutes. After that shell membrane was removed carefully, egg shells were dried in the air  
85 and weighed using an electronic balance.

#### 86 **Statistical Analysis**

87 The data generated was analyzed for two way ANOVA using factorial RBD and  
88 means were compared using Duncan's post-hoc test at P<0.05 on SPSS software (Snedecor  
89 and Cochran, 1994).

### 90 **RESULTS AND DISCUSSION**

#### 91 **Egg shell thickness (mm)**

##### 92 **Effect of dietary supplementation and bedding material**

93 The statistical analysis of variance of data revealed highly significant (P<0.01) effect  
94 of dietary supplementation and bedding materials on the overall mean egg shell thickness of  
95 Japanese quail during the experiment (Table 4). However, during 8<sup>th</sup>, 10<sup>th</sup>, 12<sup>th</sup>, 14<sup>th</sup> and 18<sup>th</sup>  
96 week of study, there was non-significant effect of bedding materials on egg shell thickness.  
97 The overall means of egg shell thickness (mm) for various dietary treatment groups were  
98 recorded to be 0.19 in T<sub>0</sub>, 0.19 in T<sub>1</sub>, 0.20 in T<sub>2</sub> and 0.20 in group T<sub>3</sub>, respectively

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99 (Table1).So, the overall study indicated that there is beneficial effect of incorporation of giloy  
 100 and ascorbic acid in the diet on egg shell thicknessof the Japanese quails.Numerically highest  
 101 egg shell thickness (mm) was found in sawdust and wheat straw (0.20) as compared to sand  
 102 (0.19) (Table2).So, the overall study indicated that there is beneficial effect of sawdust and  
 103 wheat straw on egg shell thickness of the Japanese quails.

104 The findings of the above experiment are in close agreement with Shit *et*  
 105 *al.*(2012) who conducted a trial in which a total of one hundred and twenty, sixty from each  
 106 sex (15 week old) of Japanese quail from same hatch were procured from the institutional  
 107 experimental quail farm and supplemented with L- ascorbic acid. Among the all the egg  
 108 quality traits studied, only specific gravity, egg shell weight and egg shell thickness differed  
 109 significantly ( $P < 0.05$ ) in the present study.

110 Similarly, Dhaliwal (2004) studied the effects of supplementation of vitamin C and E  
 111 on growth and reproduction were studied of Japanese quail (*Coturnix coturnix japonica*)  
 112 kept in cages. Synthetic vitamin C and E or herbal CE supplementations were tested. Egg  
 113 shell thickness increased significantly ( $P < 0.05$ ) on supplementation of vitamin E and herbal  
 114 CE.

115 Results are also similar with the findings of Karimi *et al.* (2015) who conducted a study  
 116 to determine whether dietary chromium (1200  $\mu\text{g Cr/kg}$  diet) and vitamin C (300 mg/kg)  
 117 attenuated adverse effects of heat stress on external and internal egg quality traits in Japanese  
 118 quails. They found increased ( $P < 0.05$ ) egg shell thickness when supplemented with vitamin  
 119 C and Cr-vitamin C groups.

**Table**

120 **1: Effect of dietary supplementation on Egg shell thickness (mm) at different weeks**

Supplement effect	Age in weeks									
	8W	10W	12W	14W	16W	18W	20W	22W	24W	8-24W
T <sub>0</sub>	0.17 <sup>a</sup>	0.18	0.18	0.18 <sup>a</sup>	0.18 <sup>a</sup>	0.19 <sup>a</sup>	0.20 <sup>a</sup>	0.20 <sup>a</sup>	0.21 <sup>a</sup>	0.19 <sup>a</sup>
T <sub>1</sub>	0.17 <sup>a</sup>	0.18	0.18	0.18 <sup>a</sup>	0.19 <sup>ab</sup>	0.2 <sup>ab</sup>	0.21 <sup>ab</sup>	0.21 <sup>b</sup>	0.21 <sup>a</sup>	0.19 <sup>b</sup>
T <sub>2</sub>	0.18 <sup>b</sup>	0.18	0.19	0.19 <sup>ab</sup>	0.19 <sup>b</sup>	0.20 <sup>b</sup>	0.21 <sup>bc</sup>	0.21 <sup>b</sup>	0.21 <sup>a</sup>	0.20 <sup>b</sup>
T <sub>3</sub>	0.18 <sup>b</sup>	0.19	0.19	0.19 <sup>b</sup>	0.20 <sup>c</sup>	0.21 <sup>c</sup>	0.22 <sup>c</sup>	0.23 <sup>c</sup>	0.23 <sup>b</sup>	0.20 <sup>c</sup>
SEM	0.001	0.002	0.002	0.002	0.002	0.002	0.003	0.003	0.003	0.001
	8	0	2	7	5	8	2	0	4	2

121 Means having different superscripts in a column differ significantly ( $P \leq 0.05$ )

122 **Table 2: Effect of bedding materials on Egg shell thickness (mm) at different weeks**

Supplement effect	Age in weeks									
	8W	10W	12W	14W	16W	18W	20W	22W	24W	8-24W
B <sub>1</sub>	0.17	0.18	0.18	0.18	0.19 <sup>a</sup>	0.19	0.20 <sup>a</sup>	0.20 <sup>a</sup>	0.20 <sup>a</sup>	0.19 <sup>a</sup>

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<b>B<sub>2</sub></b>	0.17	0.18	0.18	0.19	0.20 <sup>b</sup>	0.20	0.22 <sup>b</sup>	0.22 <sup>b</sup>	0.22 <sup>b</sup>	0.20 <sup>b</sup>
<b>B<sub>3</sub></b>	0.17	0.18	0.18	0.19	0.19 <sup>a</sup>	0.20	0.21 <sup>a</sup>	0.22 <sup>b</sup>	0.22 <sup>b</sup>	0.20 <sup>b</sup>
<b>SEM</b>	0.001 5	0.001 7	0.001 9	0.002 3	0.002 2	0.002 4	0.002 8	0.002 6	0.002 9	0.001 0

Means having different superscripts in a column differ significantly ( $P \leq 0.05$ )

### Interaction effect of dietary supplements × bedding materials

The statistical analysis of variance of data revealed non-significant effect of interaction between dietary supplementation and different bedding materials on egg shell thickness of Japanese quail during the entire period of study (Table 4). Furthermore, the comparison of means showed that the highest egg shell thickness was recorded in T<sub>23</sub> and T<sub>33</sub> group (Table 3). Lowest egg shell thickness was found in T<sub>10</sub>, T<sub>11</sub>, T<sub>12</sub> and T<sub>30</sub> groups (Table 3). So, the overall study indicated that there is no beneficial effect of bedding material with supplementation of both gilyo and ascorbic acid on egg shell thickness of the Japanese quails.

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**Table 3: Effect of dietary supplements × bedding materials Interaction on Egg shell thickness (mm) at different weeks**

Interaction Effect	Age in weeks									
	8W	10W	12W	14W	16W	18W	20W	22W	24W	8-24W
T <sub>10</sub>	0.17	0.18	0.19	0.18	0.19	0.19	0.20	0.19	0.20	0.19
T <sub>11</sub>	0.17	0.19	0.19	0.19	0.19	0.20	0.20	0.20	0.21	0.19
T <sub>12</sub>	0.18	0.18	0.19	0.19	0.19	0.20	0.21	0.21	0.21	0.19
T <sub>13</sub>	0.18	0.19	0.19	0.20	0.20	0.21	0.22	0.22	0.22	0.20
T <sub>20</sub>	0.18	0.19	0.19	0.19	0.19	0.20	0.21	0.21	0.22	0.20
T <sub>21</sub>	0.17	0.19	0.19	0.19	0.20	0.20	0.22	0.22	0.22	0.20
T <sub>22</sub>	0.18	0.19	0.19	0.20	0.21	0.21	0.22	0.22	0.23	0.20
T <sub>23</sub>	0.18	0.19	0.19	0.21	0.22	0.23	0.24	0.24	0.23	0.21
T <sub>30</sub>	0.17	0.18	0.18	0.19	0.19	0.19	0.20	0.21	0.22	0.19
T <sub>31</sub>	0.18	0.19	0.19	0.19	0.20	0.20	0.22	0.22	0.22	0.20
T <sub>32</sub>	0.18	0.19	0.19	0.19	0.20	0.21	0.21	0.22	0.22	0.20
T <sub>33</sub>	0.19	0.19	0.19	0.20	0.20	0.21	0.21	0.23	0.24	0.21
<b>SEM</b>	0.003 1	0.003 5	0.003 8	0.004 7	0.002 5	0.004 9	0.005 7	0.005 3	0.005 9	0.002 1

135 **Table 4 Mean sum of squares for Egg shell thickness at different weeks**

Source of variation	D F	Age in weeks									
		MEAN SQUARES									
		8W	10W	12W	14W	16W	18W	20W	22W	24W	8-24W
Supplement	3	0.0004 2**	0.000 14	0.000 10	0.000 37*	0.0005 6**	0.001 0**	0.0009 6**	0.0014 **	0.0009 4**	0.0005 6**
Bedding	2	0.0000 43	0.000 081	0.000 027	0.000 20	0.0005 7**	0.000 30	0.0012 **	0.0011 **	0.0013 **	0.0003 8**
Interaction (TxB)	6	0.0000 93	0.000 036	0.000 032	0.000 015	0.0000 43	0.000 058	0.0002 0	0.0000 57	0.0000 65	0.0000 11
Error	1 2	0.0000 38	0.000 050	0.000 058	0.000 089	0.0000 78	0.000 097	0.0001 3	0.0001 125	0.0001 4	0.0000 18

\*= significant (P≤0.05), \*\*= highly significant (P≤0.01)

137 **Egg shell weight**

138 **Effect of dietary supplementation and bedding material**

139 The overall means of egg shell weight for various dietary treatment groups were  
 140 recorded to be 1.44 in T<sub>0</sub>, 1.53 in T<sub>1</sub>, 1.55 in T<sub>2</sub> and 1.76 in group T<sub>3</sub>,  
 141 respectively (Table 5). So, the overall study indicated that there is beneficial effect of  
 142 incorporation of giloy and ascorbic acid in the diet on egg shell weight of the Japanese  
 143 quails. The statistical analysis of variance of data revealed highly significant (P<0.01) effect  
 144 of incorporation of supplements and bedding materials on mean egg shell weight of Japanese  
 145 quail throughout the experimental period (Table 8). However, during 8<sup>th</sup>, 10<sup>th</sup>, 12<sup>th</sup>, 14<sup>th</sup> and  
 146 16<sup>th</sup> week of study there was non-significant effect of bedding materials on egg shell weight  
 147 (Table 6). Numerically highest egg shell weight (1.59) was found in sawdust group as  
 148 compared to other bedding material (Table 8). So, the overall study indicated that there is  
 149 beneficial effect of sawdust on egg shell weight of the Japanese  
 150 quails. Similarly, Bardakcioglu *et al.* (2005) carried out an experiment to investigate the  
 151 effects of dietary vitamin C supplementation on some egg production traits and egg shell  
 152 quality in Japanese quails reared under high ambient temperature. There was significant  
 153 effect of vitamin C supplementation on egg shell weight. The findings of the above  
 154 experiment are in close agreement with Shit *et al.* (2012) and Karimiet *al.* (2015) in Japanese  
 155 quail.

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156 **Table 5: Effect of dietary supplementation on Egg shell weight (g) at different weeks**

Supplement effect	Age in weeks									
	8W	10W	12W	14W	16W	18W	20W	22W	24W	8-24W
T <sub>0</sub>	0.95 <sup>a</sup>	1.14 <sup>a</sup>	1.16 <sup>a</sup>	1.41 <sup>a</sup>	1.50 <sup>a</sup>	1.65 <sup>a</sup>	1.70 <sup>a</sup>	1.72 <sup>a</sup>	1.72 <sup>a</sup>	1.44 <sup>a</sup>
T <sub>1</sub>	1.06 <sup>b</sup>	1.24 <sup>b</sup>	1.26 <sup>b</sup>	1.50 <sup>b</sup>	1.65 <sup>b</sup>	1.75 <sup>b</sup>	1.76 <sup>b</sup>	1.77 <sup>b</sup>	1.76 <sup>ab</sup>	1.53 <sup>b</sup>
T <sub>2</sub>	1.09 <sup>c</sup>	1.28 <sup>c</sup>	1.31 <sup>c</sup>	1.52 <sup>b</sup>	1.62 <sup>b</sup>	1.74 <sup>b</sup>	1.79 <sup>b</sup>	1.80 <sup>c</sup>	1.80 <sup>b</sup>	1.55 <sup>c</sup>
T <sub>3</sub>	1.28 <sup>d</sup>	1.48 <sup>d</sup>	1.51 <sup>d</sup>	1.70 <sup>c</sup>	1.88 <sup>c</sup>	1.98 <sup>c</sup>	2.01 <sup>c</sup>	2.02 <sup>d</sup>	2.02 <sup>c</sup>	1.76 <sup>d</sup>
SEM	0.0089	0.0093	0.014	0.010	0.019	0.0098	0.012	0.012	0.015	0.0066

157 Means having different superscripts in a column differ significantly (P≤0.05)

158 **Table 6: Effect of bedding materials on Egg shell weight (g) at different weeks**

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Supplement effect	Age in weeks									
	8W	10W	12W	14W	16W	18W	20W	22W	24W	8-24W
B <sub>1</sub>	1.08	1.27	1.30	1.52	1.64	1.75 <sup>a</sup>	1.78 <sup>a</sup>	1.79 <sup>a</sup>	1.79 <sup>a</sup>	1.55 <sup>a</sup>
B <sub>2</sub>	1.10	1.30	1.32	1.54	1.67	1.79 <sup>b</sup>	1.84 <sup>b</sup>	1.85 <sup>b</sup>	1.85 <sup>b</sup>	1.59 <sup>b</sup>
B <sub>3</sub>	1.10	1.29	1.32	1.54	1.66	1.79 <sup>b</sup>	1.83 <sup>b</sup>	1.84 <sup>b</sup>	1.84 <sup>b</sup>	1.58 <sup>b</sup>
SEM	0.0077	0.0080	0.012	0.0086	0.016	0.0085	0.011	0.010	0.013	0.0057

159 Means having different superscripts in a column differ significantly (P≤0.05)

160 **Interaction effect of dietary supplements × bedding materials**

161 The statistical analysis of variance of data revealed non-significant effect of  
 162 interaction between dietary supplementation and different bedding materials on egg shell  
 163 weight of Japanese quail during the entire period of study (Table 8).  
 164 Furthermore, the comparison of means showed that the highest egg shell weight in T<sub>23</sub> and T<sub>33</sub>  
 165 group (Table 7). The lowest egg shell weight was found in T<sub>10</sub> group. So, the overall study  
 166 indicated that there is no beneficial effect of bedding material with supplementation of both  
 167 giloy and ascorbic acid on egg shell weight of the Japanese quails.

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168 **Table 7: Effect of dietary supplements × bedding materials Interaction on Egg shell**  
 169 **weight (g) at different weeks**

Interaction Effect	Age in weeks									
	8W	10W	12W	14W	16W	18W	20W	22W	24W	8-24W
T <sub>10</sub>	0.94	1.14	1.16	1.41	1.48	1.61	1.65	1.66	1.67	1.41
T <sub>11</sub>	1.04	1.25	1.26	1.49	1.66	1.74	1.76	1.76	1.76	1.52
T <sub>12</sub>	1.08	1.27	1.30	1.50	1.59	1.72	1.74	1.77	1.77	1.52
T <sub>13</sub>	1.28	1.46	1.50	1.70	1.88	1.97	1.99	1.99	1.97	1.75

T <sub>20</sub>	0.96	1.16	1.18	1.43	1.55	1.68	1.74	1.76	1.76	1.47
T <sub>21</sub>	1.08	1.26	1.27	1.51	1.66	1.75	1.78	1.79	1.78	1.54
T <sub>22</sub>	1.10	1.30	1.32	1.53	1.63	1.77	1.84	1.83	1.84	1.57
T <sub>23</sub>	1.29	1.50	1.55	1.72	1.87	1.99	2.03	2.06	2.04	1.78
T <sub>30</sub>	0.95	1.14	1.17	1.40	1.48	1.67	1.73	1.76	1.76	1.45
T <sub>31</sub>	1.07	1.24	1.28	1.51	1.64	1.77	1.77	1.77	1.77	1.53
T <sub>32</sub>	1.10	1.30	1.34	1.56	1.65	1.74	1.82	1.82	1.81	1.57
T <sub>33</sub>	1.30	1.50	1.50	1.70	1.90	1.99	2.02	2.03	2.06	1.78
SEM	0.015	0.016	0.024	0.017	0.033	0.017	0.022	0.020	0.026	0.011

170 **Table 8: Mean sum of squares for Egg shell weight at different weeks**

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Source of variation	DF	Age in weeks									
		MEAN SQUARES									
		8W	10W	12W	14W	16W	18W	20W	22W	24W	8-24W
Supplement	3	0.23**	0.24*	0.25**	0.18**	0.29**	0.24**	0.21*	0.21*	0.20*	0.22**
Bedding	2	0.0025	0.0022	0.0028	0.0026	0.0032	0.0074**	0.017**	0.018**	0.017**	0.0068**
Interaction (TxB)	6	0.00028	0.00044	0.00013	0.00011	0.00033	0.0011	0.0020	0.0019	0.0021	0.00039
Error	12	0.00095	0.0010	0.0023	0.0012	0.0044	0.0011	0.0020	0.0017	0.0027	0.00053

171 \*\*= highly significant (P<0.01)

172 **CONCLUSION**

173 The findings of the study indicate that supplementation of giloy and ascorbic acid in  
 174 the diet improves egg quality traits of the Japanese quails, and that there is beneficial effect of  
 175 saw dust and wheat straw as bedding material. If these bedding material used with  
 176 supplementation of both giloy and ascorbic acid improves egg shell thickness and egg shell  
 177 weight.

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