The Effect of Adding Bee Pollen on The Sexual Efficiency of Quail Males

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ABSTRACT
This study aims to determine the role of bee pollen in improving the reproductive efficiency of quail males raised at high density. The total number was 288 birds at 78 weeks old, randomly distributed to 6 experimental groups. The 1st group was placed at a rate of 21 birds / m², 2nd group was placed at a rate of 21 birds / m² and addition of bee pollen 20 g/kg feed, the 3rd group was at a rate of 21 birds / m² addition of bee pollen 30 g/kg feed, the 4th group was high density 75 birds / m², 5th group was high density 75 birds / m² and supplemented with bee pollen 20 g/kg feed, and the 6th group was high density 75 birds / m² and supplemented with bee pollen 30 g/kg feed. The results showed that adding 20g and 30g of bee pollen to the diet of 21 birds caused a significant increase in testosterone, sperm count and live sperm accompanied by an increase in the dead and deformed sperm. Furthermore, decrease in body weight. Adding 20g and 30g of bee pollen to the high density of birds resulted in a significant increase in testosterone, sperm count, and live sperm accompanied by a reduction in dead and deformed sperm and an increase in body weight. The study concluded that the high density of the birds' numbers had a negative impact on the bird's reproductive efficiency and body weight while adding bee pollen to the diet improved quail male body weight and reproductive efficiency.

Keywords: Bee pollen, High density, quails, Spermatogenesis, Testosterone.

INTRODUCTION
High bird densities are a huge stress aspect because they have an effect on the health and performance of birds under identical conditions and stress is among the main management problems in the poultry industry; physiological factors that cause stress include climate, environment, nutrition, vaccines, lighting, bird density, and transportation (Puvadolpirod and Thaxton, 2000). Poultry breeders are now under intense pressure to reduce production costs, which has resulted in increased bird density, and this strategy may be a viable option for increasing production yield. (Leandro et al., 2005). However, feed consumption declines when bird populations gradually expand over the breeding season (Goldflus et al., 1997). Krause et al. (2019) further confirmed that sex hormone levels are lowered at high density.

Any ingredient added to feed can improve the quality of egg and meat output in poultry production and rearing systems, particularly in quail farming techniques (Batkowska et al., 2018). The use of natural additives or vital plants in poultry feeding is of particular importance, and one of the most important of those natural additives is the use of bee pollen (Klarić et al., 2018). Bee workers (Apis mellifera) gather flower pollen from various plant sources to create bee pollen (Attia et al., 2014). Bee pollen generally consists of 25 to 30 % protein, 30 to 55 percent carbohydrates, 1 to 20 percent fatty acids and lipids, as well as vitamins, minerals, sterols, phenolic acids and flavonoids (Martiniakova et al., 2021). Bee pollen contains essential nutrients that have a beneficial nutritional effect on reproductive effectiveness; as mentioned by Wang et al. (2002), adding 1 and 1.5 % bee pollen improved semen quality and increased broiler chicken activity and sperm concentration. In a previous study by Salman et al. (2014), the addition of bee pollen has a role in increasing the concentration of sperm.

The aim of the study is to study the effect of bird population density and the role of adding bee pollen on the reproductive efficiency and body weight of male quail.
MATERIALS AND METHODS

1. Materials

The birds were fed from the productive ration with the addition of bee pollen obtained from the local markets of the city of Mosul, and it was collected from the pollen of the palm trees of the city of Hilla. It was given in two doses of 20 gm (Akın et al., 2021) and 30 gm mixed with the ration and according to the groups.

1.1. Birds

This research was conducted at the University of Mosul's College of Veterinary Medicine's animal house. Quail males were received from the Agricultural Research department of the Nineveh Agriculture Directorate. For this investigation, 288 seven weeks old Japanese quails were maintained per each group in a complete randomized design. The quails were kept in floor pens in an area of 1 square meter for each group throughout the research; quails were fed ad-libitum on a basal diet (National Research Council, 1994) (table 1).

<table>
<thead>
<tr>
<th>the ingredients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>wheat</td>
<td>22</td>
</tr>
<tr>
<td>Crushed yellow corn</td>
<td>42</td>
</tr>
<tr>
<td>animal protein</td>
<td>4</td>
</tr>
<tr>
<td>Vegetable oil</td>
<td>1</td>
</tr>
<tr>
<td>soybean</td>
<td>30</td>
</tr>
<tr>
<td>salt</td>
<td>0.3</td>
</tr>
<tr>
<td>limestone</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Table 1: The percentage of the components of the diet used in the Experiment

Fresh tap water was available throughout the experimental period (8th weeks), and steady light for 14 hours per day. The ambient temperature varied from 20°C to 25°C. There was a 45 - 60% humidity level range.

2. Methods

2.1. Experimental design

The quails were randomly distributed to 6 experimental groups, three of them (21 birds) were divided into three replicates, 7 birds for each replicate (3x7), and three other groups (75 birds) were also divided into three replicates, 25 birds for each replicate (3x25). The 1st group of quails was put at a rate of 21 birds/ m² (control), normal density (ND), and fed the basal diet; the 2nd group of quails was put at a rate of 21 birds/ m² and the diet addition with bee pollen (BP) 20 g/kg diet, 3rd group quails was put at a rate of 21 birds/ m² and the diet addition with BP 30 g/kg diet, 4th group high density (HD) of 75 birds/ m² fed the basal diet, 5th group high density (HD) of 75 birds/ m² and the diet supplemented with (BP) 20 g/kg diet, 6th group high density (HD) of 75 birds/ m² and the and the diet supplemented with (BP) 30 g/kg diet.

2.2. Blood samples

Blood samples were collected during the slaughter and centrifuged for 15 minutes at 3000 rpm to separate the serum. Before estimating the testosterone hormone concentrations Kit from company Monobind Inc. using the ELISA technique Competitive Enzyme Immunoassay, serum samples were kept at -20°C.

2.3. Internal organ weights

Testis weight, foam gland weight, and body weight were calculated as organ weight/100 g body weight. (Matty and Hassan, 2020).

2.4. Spermatogenesis

The abdomen was opened immediately after the bird was decapitated, and the ductus deferens were squeezed from the epididymis duct to the base of the duct. The sperms were then diluted with eosin stain and normal saline solution, and spermatogenesis values such as sperm concentration and percentage of live, dead, and abnormal sperms were determined (Tarif et al., 2013).

2.5. Statistical analysis

A one-way analysis of variance was used to analyze the data (ANOVA). The Duncan Multiple Range Test at P ≤0.05 was used to determine the significance of differences between means. (Steel et al., 1997).

RESULTS

The data presented in Table 2 shows that adding 20g and 30 g of bee pollen to the ration of 21 birds produced a significant rise (P ≤0.05) in the testosterone hormone compared with the control group. The consequences of the recent study documented that the promotion in the number of birds by 75 birds / m² (HD) initiated a significant reduction (P ≤0.05) in the testosterone hormone compared with the control group, and adding 20g and 30 g of bee pollen to the diet of the high density group led to a significant rise (P ≤0.05) in the testosterone hormone linked with the high density group of birds that were given a diet without of bee pollen.

Findings from the recent parameter show that adding 20g and 30 g/kg of bee pollen to the diet of 21...
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Birds was not significantly change their ultimate live body weight compared to the control group. The increment in bird numbers of 75 quails/m² showed a significant decline (P≤0.05) in body weight against the control value. Adding 20 and 30 g of bee pollen to the ration of the bird high density group led to a dramatic elevate (P≤0.05) in the final body weight as opposed to the high density group of birds that weren’t given bee pollen.

The results did not record a significant difference (P> 0.05) in the weight of the right, left testis, and foam glands when adding 20 g and 30 g of bee pollen to the diet of 21 bird groups compared with the control group. Furthermore, the high density of bird numbers also did not lead to a significant difference in the weight of the right and left testis. Compared to the control group, the Foam gland and adding 20 g and 30 g of bee pollen to high density birds did not significantly affect the weight of the right and left testis. Foam gland compared with the high density group of birds that were not given bee pollen.

The statistical analysis of current results demonstrated in Table 3 did not record a significant difference (P>0.05) in sperm count, the percentage numbers of live and dead. Abnormal sperms for the group of 21 birds and the addition of bee pollen at 20 and 30 g/kg diet compared with the normal control value (the number of birds was 21 birds and they were given a standard diet). In comparison, the increase in the number of birds density of 75 birds/m² (HD) led to a significant decrease (P≤0.05) in the number of sperms and the percentage number of live sperms was accompanied by a significant increase (P≤0.05) in the percentage number of dead and deformed sperms compared with the control group.

The results demonstrated the addition of bee pollen 20 g and 30 g/kg diet to the high density birds (75 birds/m²) produced a significant increase in the sperm count and the percentage of live sperm with a significant decline in the percentage of dead and abnormal sperm in comparison with the high density group of birds (75 birds/m²).

Table 2: Effect of high density of bird numbers and the addition of bee pollen to the diet on the concentration of testosterone, body weight, weight of right and left testis and foam gland weight of quail males (Mean ± SE):

<table>
<thead>
<tr>
<th></th>
<th>Testosterone ng/ml</th>
<th>Body weight (g)</th>
<th>Right testicle weight g/100g BW</th>
<th>Left testicle weight g/100g BW</th>
<th>Foam gland weight g/100 g BW</th>
</tr>
</thead>
<tbody>
<tr>
<td>(G1) Control group</td>
<td>5.12 ±0.64&lt;sup&gt;c&lt;/sup&gt;</td>
<td>201.83±1.72&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.70 ±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.82 ±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.13 ±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(G2) added bee pollen 20g/kg</td>
<td>11.42 ±0.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>203.00±0.85&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.50 ±0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.52 ±0.41&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.11 ±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(G3) added bee pollen 30g/kg</td>
<td>8.78 ±1.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>202.16±0.98&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.58 ±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.64 ±0.09&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.01 ±0.06&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>(G4) High density group</td>
<td>2.19 ±0.43&lt;sup&gt;d&lt;/sup&gt;</td>
<td>198.50±0.80&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.38 ±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.51 ±0.06&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.06 ±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(G5) High density group + bee pollen 20g/kg</td>
<td>9.30 ±0.69&lt;sup&gt;a&lt;/sup&gt;</td>
<td>208.16±0.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.26 ±0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.47 ±1.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.91 ±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(G6) High density+ bee pollen 30g/kg</td>
<td>8.03 ±1.60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>207.66±0.98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.37 ±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.48 ±1.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.21 ±0.09&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values using different letters in the column are significantly different P<0.05.
Table 3: Effect of high density of bird numbers and the addition of bee pollen to the diet on the spermatogenesis of quail males

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean ± SE</th>
<th>Sperm number</th>
<th>Live sperm</th>
<th>Dead sperm</th>
<th>Sperm deformity</th>
</tr>
</thead>
<tbody>
<tr>
<td>(G1) Control group</td>
<td></td>
<td>40.66 ± 6.11a</td>
<td>47.33 ±1.92b</td>
<td>52.66 ± 1.92b</td>
<td>28.83 ± 3.52ab</td>
</tr>
<tr>
<td>(G2) add pollen 20g/kg</td>
<td></td>
<td>45.66 ± 4.94a</td>
<td>51.16 ± 3.03ab</td>
<td>48.83 ± 3.03bc</td>
<td>20.50 ± 4.10b</td>
</tr>
<tr>
<td>(G3) add pollen 30g/kg</td>
<td></td>
<td>46.83 ± 6.30a</td>
<td>49.32 ± 1.08ab</td>
<td>50.66 ± 1.08b</td>
<td>23.83 ± 3.72b</td>
</tr>
<tr>
<td>(G4) High density group 75 birds/m²</td>
<td></td>
<td>24.50 ± 2.32c</td>
<td>32.83 ± 2.48c</td>
<td>67.16 ± 2.48a</td>
<td>35.50 ± 3.45a</td>
</tr>
<tr>
<td>(G5) High density +pollen 20g/kg</td>
<td></td>
<td>35.00 ± 2.36ab</td>
<td>56.83 ± 4.84a</td>
<td>41.50 ± 4.03c</td>
<td>27.50 ± 2.36ab</td>
</tr>
<tr>
<td>(G6) High density +pollen 30g/kg</td>
<td></td>
<td>32.33 ± 3.23b</td>
<td>52.00 ± 2.67ab</td>
<td>48.00 ± 2.67bc</td>
<td>23.50 ± 2.71b</td>
</tr>
</tbody>
</table>

Values using different letters in the column are significantly different P<0.05.

DISCUSSION

According to the latest findings in the present study, a significant drop in testosterone hormone was caused by the HD (75 birds per square meter) habitat. Our findings corroborated those of Fahmy et al., (2005), who claimed that the high bird population density caused the testicles to formally atrophy and the testosterone level sharply declined. The cause of low testosterone hormone levels is related to stress brought on by the high bird density, which has a detrimental impact on testosterone concentration, semen volume, sperm concentration, and motility (Elnagar, 2010).

The present results recorded a significant increase in testosterone when adding 20 and 30 g of bee pollen/kg ration to the group of 21 birds/m² compared to the control group, as well as when adding 20 and 30 gm of bee pollen/kg ration to the group density of 75 birds/m² compared with the high density group that did not bee pollen given. The current results corroborated those of Abdulwahid and Hassan, (2019), who found that male rabbits treated with zinc sulfate had significantly higher levels of the hormone testosterone than those in the control group. Zinc is a superior antioxidant essential for maintaining healthy immune systems and protein synthesis. According to Erdogan et al., (2022), bee pollen contains 17.7 56.2 mg/kg zinc. This can be attributed to phenolic compounds, phytoestrogens that are metabolized in the cell to enterolactone and introdiol, which have estrogen-like effects (Zingue et al., 2017). These compounds can also have an impact on the hypothalamus and adrenohypophysis, which in turn affect the production of LH and FSH, which in turn stimulates the gonads to increase testosterone production and spermatogenesis (Pineda and Dooley, 2003).

The current study demonstrated a significant decrease in the final body weight of the high density group (75 birds/m²) compared to the control group. The current study’s findings are consistent with those of earlier investigations by Cengiz et al., (2015); El-Tarabany (2016), which demonstrated a reduction in ultimate body weight. At a significant quail density, the significant decrease in final body weight may contribute to the corticosterone-raising effect (caused by stress due to the high population density of birds), which markedly reduces food intake and reduced body weight in poultry (El-Kazaz and Hafez, 2020).

The present results showed a considerable rise in final body weight when adding 20g and 30g of bee pollen to the diet of high density number (75 bird/m²); these outcomes are strong consistency with those of Fazayeli-Rad et al., (2015), who found that broiler chicks fed bee pollen for 6 weeks caused steady increased in body weight gain, the nutritional profile of bee pollen combined with various phenolic components, organic materials for antioxidant activities, and protection of the bird’s health may be the cause of the improved body weight gain (Šarić et al., 2009).

The current findings revealed a decrease in the number of sperm and the percentage of living sperm while significantly increasing the number of dead and
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deformed sperm. In high density of birds (75/m²), this result was in agreement with Sikka, (2001) as it was shown that the stress resulting from the density of bird numbers led to a decrease in the number and movement of sperm and an increase in the percentage of dead sperm in semen of rooster. The decline in sperm concentration could be attributed to stress caused by high bird density, which lowers testosterone levels. (Elnagar, 2010). Our study supports this, showing the negative impact of bird population density on testosterone levels, which in turn influences the count of sperm.

The current results show that adding 20g and 30g of bee pollen to high density birds significantly increases the number of sperm and the percentage of live sperm and significantly decreases the percentage of dead and malformed sperm. Similar research to those of researcher Abuoghaba (2018), who reported that bee pollen treatment in roosters raised semen volume, live sperm and motility, and sperm concentration and reduced the percentage of dead sperm and deformed sperm, the rise in sperm concentration in the bee pollen treated group can be due to an increase in luteinizing hormone concentration, which stimulates testosterone release from Leydig cells and increases the creation of sperm from germ cells (Abuoghaba, 2018).

CONCLUSION

The high density of birds had a negative effect on the efficiency of bird reproduction. Adding bee pollen to the diets of 21 and 75 quail males resulted in increased body weight and testosterone levels and improved reproductive efficiency.

Acknowledgments

We would like to express our appreciation and gratitude to the College of Veterinary Medicine at the University of Mosul, particularly the Physiology, Biochemistry, and pharmacology department, for providing the facilities necessary to complete this research.

Conflict of interest

The authors declare that they are not involved in any potential conflicts of interest.

REFERENCES


