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Research Article

The Effects of Hot Pepper Seeds Added to the Diet in Quails on the Morphology of the Oviduct and Ovary

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ABSTRACT

The aim of this study is to reveal how red-hot pepper seeds added to the diet in quails change ovarian follicle dynamics and the morphological structure of the oviduct. For this reason, a total of 48 female Japanese quails (*Coturnix coturnix Japonica*) aged 14 weeks were used as a material. While red hot pepper (*Capsicum annuum L.*) seeds were added to the diet of the animals in the experimental group at the rate of 2%, 4%, and 6%, it was not added to the control group. At the end of the study, while the number of white follicles showed a statistically significant increase in all groups compared to the control group (P<0.05), a significant increase was recorded in the number of yellowish follicles in the groups in which 2% and 4% red pepper seeds were added to the diet compared to the control group. Also, the correlation analysis revealed that there was a positive correlation between the yellowish follicle and the white follicle. In the present study, it was observed that there was a negative correlation between magnum length and isthmus length (r=-0.369, P=0.019), while there was a positive correlation between the total length of the oviduct and yellow follicle diameter (r=0.335, P=0.034). Consequently, it was concluded that the addition of red-hot pepper (2% and 4%) seeds to the diet may increase egg yield in quails.

***Keywords**: Capsicum annuum L., Coturnix coturnix Japonica, folliculogenesis, oviduct.*

Bıldırcınlarda Rasyona Eklenen Acı Biber Tohumunun Yumurta Kanalı ve Yumurtalık Morfolojisi Üzerine Etkileri

ÖZET

Bu çalışmanın amacı, bıldırcınlarda rasyona eklenen kırmızı-acı biber tohumlarının ovaryum folikül dinamikleri ve oviduk morfolojik yapısı üzerine etkilerini ortaya koymaktır. Bu amaçla, materyal olarak 14 haftalık toplam 48 adet dişi Japon bıldırcını (*Coturnix coturnix Japonica*) kullanıldı. Kırmızı acı biber (*Capsicum annuum L.*) tohumları deney grubundaki hayvanların rasyonlarına %2, %4 ve %6 oranlarında katılırken, kontrol grubundaki hayvan rasyonlarına katılmadı. Çalışma sonunda kontrol grubu dışındaki tüm gruplarda beyaz folikül sayısında istatistiksel olarak anlamlı bir artış gözlendi (P<0,05). Rasyonuna %2 ve %4 oranında kırmızıbiber tohumları eklenen gruplar kontrol grubuna göre sarımsı folikül sayısında anlamlı bir artış kaydedildi. Ayrıca yapılan korelasyon analizi, sarımsı folikül ile beyaz folikül arasında pozitif bir korelasyon olduğunu ortaya koydu. Bu çalışmada magnum uzunluğu ile isthmus uzunluğu arasında negatif korelasyon olduğu görülürken (r=-0,369, P=0,019), oviduk kanalı toplam uzunluğu ile sarı folikül çapı arasında pozitif korelasyon olduğu görüldü (r=0,335, P=0,034). Sonuç olarak, kırmızı acı biber (%2 ve %4) tohumlarının diyete eklenmesinin bıldırcınlarda yumurta verimini artırabileceği kanısına varıldı.

***Anahtar Kelimeler**: Capsicum annuum L., Coturnix coturnix Japonica, folikülogenezis, ovidukt.*

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Introduction

Industrial poultry farming is one of the most effective ways to supply the demand for animal protein increasing in parallel with the growth in world population (Wallinga et al., 2022). The ovary and the oviduct are the two primary organs in avians that are responsible for reproductive performance. The avian oviduct is divided into four regions: the infundibulum where fertilization takes place; the magnum where egg white (albumin) and chalazae form; the isthmus where the inner and outer shell membranes form; and the uterus where the shell forms (Assersohn et al., 2021).

Scientists have been studying many herbal and nature-identical compounds as feed additives in recent years due to some objectives such as consumer demands in the poultry sector, avoiding antibiotics and anti-coccidiosis, and preserving the ecological balance by reducing the use of maize and soy (Rossi et al., 2020). One of the most extensively studied products in this field is red hot pepper (*Capsicum annuum L*). Capsaicin (8-methyl-N-vanillyl-6-nonenamide) is the active ingredient in hot pepper (Srinivasan, 2016). In addition to the different pharmacological properties of hot pepper or capsaicin such as analgesia (Kim et al., 2003), antineoplastic (Clark and Lee, 2016), antioxidant (Chaudhary et al., 2019; Li et al., 2019), immune modulator (Antonious, 2018), anti-obesity (Zsiboras et al., 2018), antibacterial (Careaga et al., 2003), and DNA protective (Oğuzkan et al., 2018), and hormone profile changes reported in avian (Erdost et al., 2006), anti-helmentic (Gentiles et al., 2019), anti-coccidial (Lozada-Ortiz et al., 2022) and antibiotic (Soliman and AlAfifi, 2020; El-Hack et al., 2022) properties, it also has properties that improve ovarian antioxidant capacity, egg production (Liu et al., 2021a) and meat quality (Liu et al., 2021b), and regulate lipid metabolism (Puvača et al., 2019).

When the studies in which red hot pepper or capsaicin was used as a feed additive in avian diets were examined (Ozfiliz, 2002; Ozer et al., 2005; Daş et al., 2022), none of the studies on the effects of red hot pepper or capsaicin on ovary and oviduct, which are the organs mainly responsible for egg yield, were found. When the seeds of red-hot pepper, which is widely produced in Turkey, are used as a feed additive in poultry, no information is available about the morphological changes it causes in the genital system organs. This study aims to reveal the changes caused by seeds of red hot pepper added to the diet as a feed additive on quail ovarian follicle dynamics and the morphological structure of the oviduct. Besides, the first findings to be obtained are intended to guide other studies to be carried out in this field and contribute to the literature.

Materials and Methods

Animal Material

In the animal material of the study, 48 female Japanese quails (*Coturnix coturnix Japonica*) at 14 weeks of age were used. The present study was conducted after ethical approval (date 22/03/2022, session 2022/003, decision 01-06) obtained from Harran University Animal Experiments Local Ethics Committee.

All stages of the study were carried out at the Avian unit, the Faculty of Veterinary Medicine at Harran University. During the experiment (14 weeks), the subjects were given feed and drinking water *ad libitum*. The animals were fed with isocaloric and isonitrogenous diets. The diet was prepared based on maize and soya. The subjects in the groups were kept in daylight and artificial lighting with 16-hour light and 8-hour darkness cycles in their henhouses. The conditions such as feeding and lighting were maintained uninterruptedly for 14 weeks. The ambient temperature was kept in the range of 18-22 °C, which is in the thermal neutral zone suitable for avian.

Formation of the Study Groups

The subjects were randomly divided into four groups according to the hypothesis of the study. No additive was included in the diet of the first group (Group Control, n=12). Red hot pepper (*Capsicum annuum L*.) seeds were added to the diets of the experimental groups at the rate of 2% in the second group (Group 2%, n=12), 4% in the third group (Group 4%, n=12) and 6% in the fourth group (Group 6%, n=12).

Provision of red hot pepper seeds

Red hot pepper (*Capsicum annuum L*.), which holds the Certificate of Geographical Indication Registration as Şanlıurfa Isot Pepper since 29/01/2001 with registration number 33 by the Turkish Patent Institute, was employed in the study. The seeds that stuck to the placenta of this pepper were sorted out, dried and stored in closed boxes to be added to the diet.

Morphological Assessment of Oviduct and Ovary

At the end of the experimental period of 14 weeks, ovaries and oviducts were resected from the slaughtered without euthanasia quails. The follicles in the ovaries of quail were counted and recorded as follows: those with white color and 1-4 mm diameter were called "white follicles", those with yellowish color and a 5-8 mm diameter were called "yellowish follicles" and those with yellow color and 9-40 mm diameter were called "yellow follicles" as reported by Anna (2021). The widths of the yellow follicles in the resected ovaries and the morphological length and width of the oviduct sections were digitally measured in mm through ImageJ software by photographing the genital system of each subject from a fixed distance (Apple iPhone 11, 12 MP. 3024x4032, Wide Camera-26 mm ƒ1.8) and saving the photograph as a HEIF file. The length unit on the ruler placed next to the specimen in the photograph was used for calibration of the software in mm.

The data obtained in the present study were statistically analyzed using the Statistical Package for the Social Sciences 24.0 package program (IBM SPSS Statistics®,

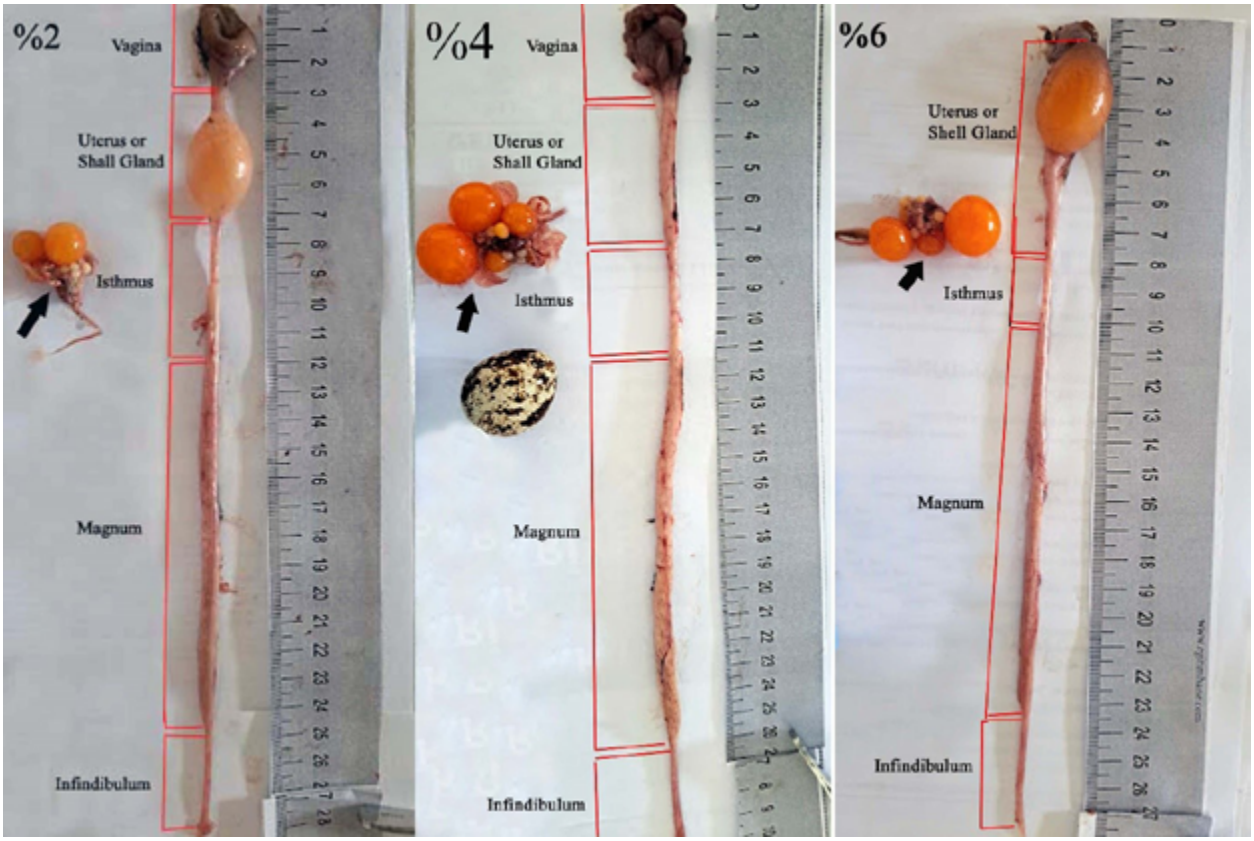

Figure 1. Group %2, Group %4, Group %6 arrow: ovarian follicles.

Chicago, IL, USA). Shapiro-Wilk test indicated that the values were normally distributed. One-way ANOVA test was used to determine whether or not there was a difference between the groups in terms of number, width and length, and the statistical significance of the difference between the groups was measured by the Duncan's test. Also, the Pearson's correlation analysis was used to determine the correlation between the measured values in all groups. The difference between the groups was considered as statistically significant when the P value was ≤0.05 (Heiberger and Neuwirth, 2009).

Results

In the study, the number of follicles (white, yellowish, yellow) of the quails in the control group, to which nothing was added to the diet, and of those in the groups to which red hot pepper (*Capsicum annuum L.*) seeds were added to the diet at the rates of 2% (Group 2%), 4% (Group 4%) and 6% (Group 6%) (Figure 1) as well as the mean diameter of yellow follicles, infundibulum, magnum and isthmus lengths and widths (Figure 2) and the statistical significance of the difference of these values between the groups are given in detail in Table 1.

In the present study, the numbers of white, yellowish and yellow follicles of the ovarian follicular activity for Groups Control, 2%, 4% and 6% were measured as 14.50±0.58, 17.10±1.02, 18.20±0.53 and 16.90±0.97 for white follicles, 1.20±0.13, 4.50±0.56, 3.60±0.69, and 2.60±0.43 for yellowish follicles, and 2.60±0.16, 2.50±0.17, 2.80±0.20, and 2.70±0.15 for yellow follicles, respectively. While the number of white follicles showed a statistically significant increase in all groups compared to the control group ($P<0.05$) the number of yellowish follicles had a significant increase in the groups 2% and 4% compared to the control group.

The correlation analysis indicated a positive correlation between the yellowish follicle and the white follicle. In the present study, there was a negative correlation between magnum length and isthmus length ($r=-0.369$, $P=0.019$), and a positive correlation was determined between the total length of the oviduct and diameter of yellow follicle ($r=0.335$, $P=0.034$) (Table 2).

Discussion

In the present study, the highest number of white follicles in relation to ovarian follicular activity was measured in Group 4%; whereas, the highest number of yellowish follicles was determined in Group 2%. Although the highest number of hierarchical yellow follicles, which are potential eggs, was obtained in Group 4%, the difference was not statistically significant. These findings are compatible with the studies (Ozer et al., 2005; Liu et al., 2021b) reporting that follicle growth and development of reproductive organs in avians is accelerated by red hot pepper or capsaicin. In the present study, it was thought that the reason for the increase in the number of quail follicles induced by hot pepper seeds was related to the changes in the number and size of the epithelial layer and theca cells surrounding the follicle in the avian ovary as reported by Ozer et al. (2005). The histological reason why this difference was observed was considered to be

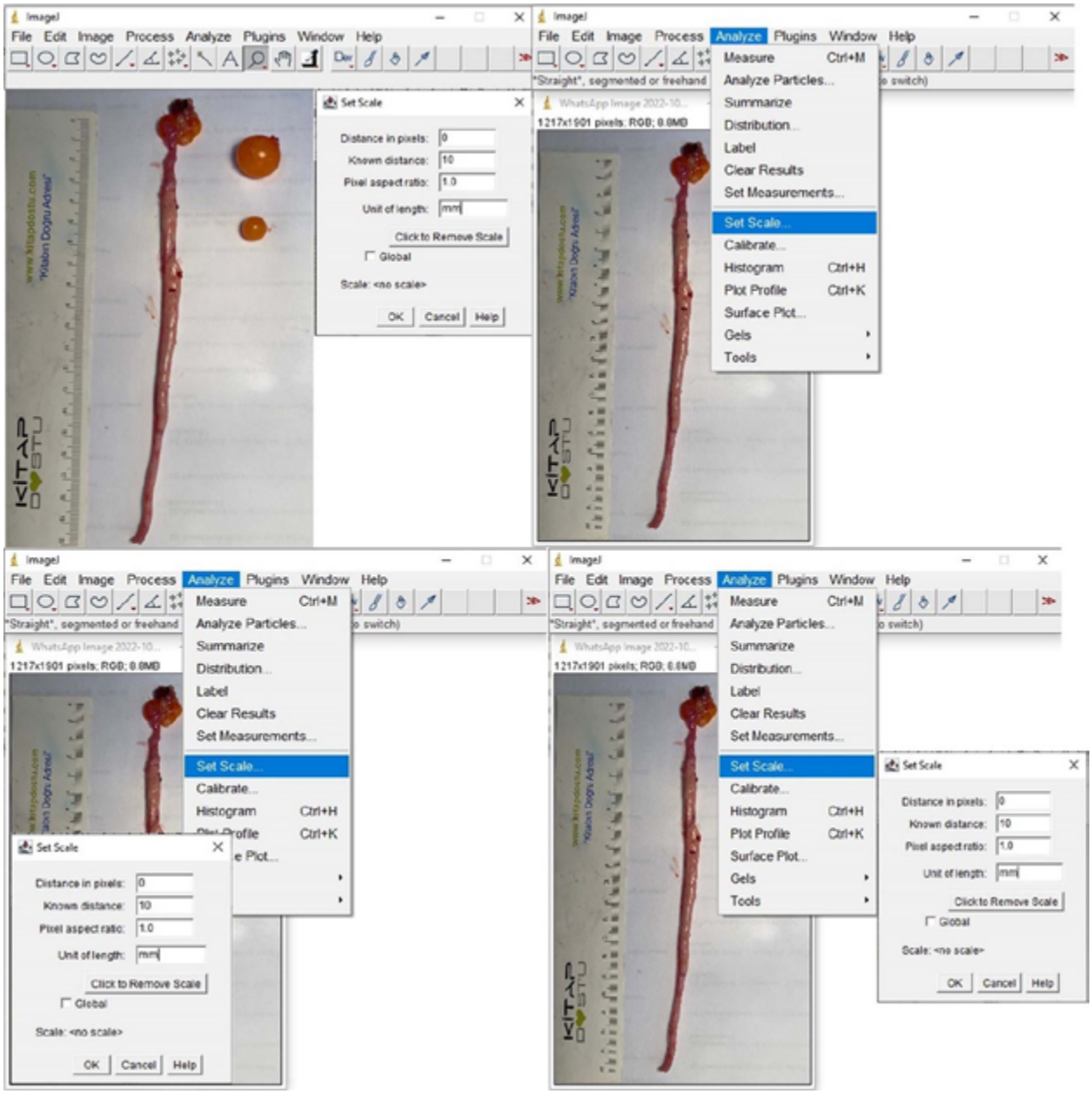

Figure 2. Group control. Digitally measurement of the genital system with ImageJ software.

due to the protective effect of capsaicin on follicles from apoptosis and atresia (Zik et al., 2010; Güler and Zik, 2018). Liu et al. (2021b) argued that capsaicin increased follicular growth and maturation in avians by activation of TRPV4 (Transient Receptor Potential Vanilloid) and calcium (Ca^{++}) signaling pathway in the ovary along with improvement in ovarian antioxidant capacity. It was considered that this condition stated by Liu et al. (2021b), may be another reason for the increased follicular activity in the present study.

Among the study groups in the present study, a significant increase was measured in the number of yellowish follicles in the group 2% compared to the group 6%. Such a high result in follicular development is compatible with the finding reported by Zik et al. (2010) that low-dose capsaicin protects ovarian follicles from apoptosis and stimulates follicular development. Likewise, the study by Güler and Zik (2018), which examined the proliferative and apoptotic effects of different doses of capsaicin on granulosa cells in the avian ovary, also reported that low-dose capsaicin treatment had positive effects on avian folliculogenesis. Alatriste et al. (2013) reported that high-dose capsaicin may affect steroidogenesis by damaging the hypothalamus-pituitary-ovarian pathway with its neurotoxic properties. In the present study, the reason why the number of yellowish follicles decreased as the dose of capsaicin increased was considered to be this effect reported by Alatriste et al. (2013).

Also, the lengths of the infundibulum, magnum and isthmus and the width of the anatomical midpoint were individually measured to assess how hot pepper seeds added to the diet affect the morphology of the oviduct in quails. The magnum length measured under this scope was significantly longer in Group 6% compared

Table 1. Comparison of the difference of measured values between groups (as Mean±SE).

	Group Control (n=12)	Group 2% (n=12)	Group 4% (n=12)	Group 6% (n=12)	P
BW (g)	232.93±6.74	230.05±8.17	230.33±3.75	221.22±5.90	0.589
In. L (mm)	35.86±3.48	37.79±4.19	36.67±3.50	41.64±4.40	0.735
In. W (mm)	3.47±0.21	3.37±0.25	3.64±0.29	3.96±0.49	0.608
Mg. L (mm)	147.76±5.57^{bc}	137.16±3.69^{c}	151.14±3.61^{b}	167.11±5.24^{a}	0.001
Mg. W (mm)	6.23±0.19	6.20±0.30	6.82±0.26	6.14±0.38	0.317
Is. L (mm)	59.30±1.86	70.53±4.91	65.46±2.57	60.30±3.56	0.095
Is. W (mm)	4.28±0.15	4.03±0.18	4.70±0.21	4.37±0.19	0.101
TL (mm)	242.92±7.26	245.48±7.30	253.28±7.06	269.05±7.01	0.060
AW (mm)	4.66±0.10	4.54±0.11	5.05±0.11	4.82±0.27	0.171
WF	14.50±0.58^{b}	17.10±1.02^{a}	18.20±0.53^{a}	16.90±0.97^{a}	0.019
YWF	1.20±0.13^{c}	4.50±0.56^{a}	3.60±0.69^{ab}	2.60±0.43^{bc}	<0.0001
YF	2.60±0.16	2.50±0.17	2.80±0.20	2.70±0.15	0.641
AYFD (mm)	14.89±0.20	14.93±0.57	15.61±0.54	16.25±0.48	0.151

$^{a, b, c}$Between groups with different letters in the same column mean difference is significant. BW: Body Weight, In.: Infundibulum, Mg.: Magnum, Is.: Isthmus, L: Length, W: Width, TL: Total Length, AE: Average Width, WF: White Follicle, YWF: Yellowish Follicle, SF: Yellow Follicle, AYFD: Average of Yellow Follicle Diameters.

to the other groups. This finding, which has no meaning at first consideration, turns out to be significant in the correlation analysis in the present study. The results of the present study indicated a negative correlation between magnum length and isthmus length (r=-0.369, P=0.019). When the significant correlations between the number of ovarian follicle types and the parts of the oviduct were examined, a positive correlation was measured between the number of yellowish follicles and the length of the isthmus (r=0.317, P=0.046). When combined with these findings in the present study, which is compatible with the results reported by Yıldırım et al. (2022), it can be asserted that as the number of yellowish follicles decreases, so will the length of the isthmus, and the length of the magnum will increase in parallel with the decrease in the length of the isthmus. This might be due to the anatomical reflection of follicular activities on the oviduct, a hollow and dynamic

Table 2. Correlation of measured values.

	In. L	In. W	Mg. L	Mg. W	Is. L	Is. W	TL	AW	WF	YWF	YF	AYFD
BW	.133	-.077	-.372*	-.094	.001	-.051	-.207	-.120	-.022	.070	.049	.014
In. L	1	-.248	.330*	.317*	-.014	-.043	.739**	.009	.033	.010	.150	.212
In. W		1	.374*	.063	-.117	.144	.096	.710**	-.289	-.226	-.122	.006
Mg. L			1	.190	-.369*	.152	.729**	.396*	-.176	-.298	.172	.147
Mg. W				1	.102	.108	.348*	.645**	-.097	-.086	.095	.067
Is. L					1	.120	.193	.030	.136	.317*	.075	.255
Is. W						1	.147	.523**	.276	.038	.039	.198
TL							1	.311	-.050	-.065	.238	.335*
AW								1	-.131	-.175	-.007	.115
WF									1	.564**	.128	.014
YWF										1	-.181	.133
YF											1	.293

BW: Body Weight. In.: Infundibulum. Mg.: Magnum. Is.: Isthmus. L: Length. W: Width. TL: Total Length. AE: Average Width. WF: White Follicle. YWF: Yellowish Follicle. SF: Yellow Follicle. AYFD: Average of Yellow Follicle Diameters. * (P<0.05). ** (P<0.01).

organ, in quails and it was considered that the magnum section of the oviduct was more intensely affected by follicular activities morphologically. When the results are analyzed, our opinion is supported by the fact that Group 2%, which had the highest mean number of yellowish follicles (4.50±0.56), also had the shortest magnum length (137.16±3.69). The present study also revealed a positive correlation between total oviduct length and yellow follicle diameter (r=0.335, P=0.034), which supports our opinion that ovarian activities would affect oviduct morphology. Hot pepper seeds in the diet were found to increase ovarian activities at certain doses and to a certain extent. Therefore, it was considered that the oviduct parts also changed morphologically in relation to the numerical changes indicating ovarian follicular activity such as yellowish follicles depending on the ratio of the additive to the diet. While a correlation was found between the length and width of the infundibulum and the total length (r=0.739, P=0.000) and mean width (r=0.710, P=0.000) of the oviduct, no significant correlation was measured between the infundibulum and ovarian follicular activities. This remarkable finding is considered to be associated with the fact that ovarian follicular activity is more related to the morphological changes of some oviduct segments, such as the isthmus, whereas the infundibulum is relatively less correlated with ovarian follicular activity. Besides our conclusion, the correlation of the infundibulum with the total length and width of the oviduct suggests that the infundibulum may be more dynamic than the other oviduct parts, which may affect the total length and average width of the oviduct. Consequently, a negative correlation was observed between the magnum and isthmus of oviduct segments in quail and it was determined that the length of the oviduct increased as the ovarian yellow follicle diameter increased. A very strong correlation was measured between the length and width of the infundibulum and the total length and average width of the oviduct. When the ovarian follicle dynamics of quails were examined, it was observed that the best results were obtained in terms of white follicle number when 4% of red hot pepper seeds were added to the diet and in terms of yellowish follicle number when 2% of red hot pepper seeds were added to the diet.

Conclusion

These results suggested that the addition of 2% and 4% of red hot pepper seeds to the diet may increase egg yield in quails. In the present study was observed, red hot pepper or capsaicin increase egg yield and was supported to the follicular activity in the ovary. Based on the results of the present study, it was concluded that further comprehensive and molecular studies to be carried out in this field may shed light on how red hot pepper seed affects the mechanism of egg yield increase.

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Conflict of interest

The authors have any conflict of interest.

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