# Effect of Natural Liquid Oreganum on Physiological Performance in Stressed Laying Japanese Quails Exposed to Force Molting

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***Abstract.*** *This study aimed to study the effect of oreganum on forced molting in laying Japanese quails to improve the physiological performance and restore the reproductive capacity of females. A total of 120 female Japanese quails (Coturnix coturnix Japonica) aged 18 weeks were divided randomly into six groups: group A – pre-molting from weeks 18 to 19; group B – supplemented with oregostim at a dose of 0.3 mL\1000 mL; group C (molting from weeks 20 to 22) – fed a restricted diet; group D – fed a limited diet with oregostim at the same dose and post-molting from week 23 to week 37; group E – fed a basal diet; and group F – fed a basal diet and treated by oregostim. The results showed that the molting group caused a significant reduction in the weight of body and ovary in group C and group D, while the weights were restored in group E and group F relative to other groups. Besides, there was a significant decrease of WBCs and RBCs count in group C and group D with a significant elevation in group E and group F compared with group A and group B. The percentage of egg production and large follicle number in group C and group D decreased significantly compared with group A and group B, while they restored to normal values in group E and group F compared with group A and group B and showed a significant increase as compared with group C and group D, respectively. We concluded from this study that oregostim does not have adverse effects on the molting period. Thus, the standard diet supplemented with oregostim was considered as a better molting technique.*

## Introduction

Force molting is a procedure of inducing molting to regenerate feathers and egg production at a particular time (Maiorano et al., 2011). It also improves the economic value by temporarily delaying the broiler productive lifespan or by temporarily delaying the egg production in laying hens (Brake, 2023). Molting is a physiological process in avian species that happens naturally and includes the replacement of old feathers with new (Huss, 2008). Numerous approaches have been used to induce molting such as using a restricted diet by feeding only grain with insufficient minerals and essential vitamins such as sodium or calcium, aluminium, zinc and iodine (Khan et al., 2011). The period of the reproductive and productive lives of birds is prolonged by force molting (Heryanto et al., 2016). The biggest significance of forced molting is the regeneration of the reproductive system, which increases the tissue efficacy, gonadal growth, and loss of adipose tissue (Narinc, 2013). The common uses of force molting include either a photoperiod stage, fasting or a combination of both (Heryanto et al., 2016). The stress phase eliminating period of feeding is followed by a recovery phase when birds receive a restricted diet of protein or total feed keeping the body weight but not resuming egg laying (Huss, 2008). Oregano is a well identified essential aroma having antioxidant properties and antioxidant activity such as thymol and carvacrol (Drăgan et al., 2008). The oregano extracts contain carvacrol or thymol (Zheng et al., 2009). Oreganum is an essential oil with antibacterial activities. It acts as an antioxidant and promotes growth. It increases feed intake by stimulating the animal's sense of hunger. Furthermore, it replaces and cleans the gut lining, which boosts the animal's health by reestablishing absorbent in the gut surface (Zhang, 2021). Studies on the applications of the product in the form of powder or leaves on production parameters and metabolism are limited (Ampode et al., 2022; Bayram and Akkaya, 2018).

The aim of this study is to determine the effect of natural liquid oreganum on physiological performance in stressed laying Japanese quails exposed to force molting by resting the reproductive system for a certain period to improve its physiological performance and restore the hens' reproductive ability.

## Materials and Methods

### *Quails*

The experimental quails and laboratory analyses were supported by an animal house and laboratories of the Veterinary Medicine Department of the University of Mosul. The study included 120 female Japanese quails (Coturnix coturnix Japonica), 18 weeks old with an average body weight ranging from 180.33 ± 36 to 200 ± 32 g purchased from al Ebaa Research Centre of the Ministry of Agriculture. The hens were preserved in floor birdcages, and food and water were provided ad libitum. Food elements and nutrient levels are presented in accordance with the

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National Research Council (NRC) recommendations (Maty and Hassan, 2020).

### *Experimental design*

Female laying hens were divided and distributed randomly into six equal groups A, B, C, D, E and F. All groups were fed a basal diet for one week for adaptation before inducing molting. The animals were kept under the same light 16 hours a day, and a water rule was ad libitum. The duration of the experiment was divided into three periods:

1. **Pre-molting period** – from week 18 to 19:

**(A) Control group**;

**(B) Oregostim group** was given oreganum at a dosage (0.3 mL \ 1000 mL water) in water.

*These two groups were fed a basal diet ad libitum.

2. **Molting period** – from week 20 to 22:

**(C) Control group**;

**(D) Oregostim group** was given oreganum at a dosage (0.3 mL \ 1000 mL water) in water.

*These two groups were fed a restricted diet ad libitum.

The restricted diet was given to the hen supplemented according to Younis (2019).

3. **Post-molting period** – from week 24 to 37:

**(E) Control group;**

**(F) Oregostim group was** given oreganum at a dosage (0.3 mL \ 1000 mL water) in water.

*These two groups were fed a basal diet ad libitum.

### *Treated material*

Oreganum product was made from 100% of pure essential oil and manufactured by Anpario Company in the UK. Oregostem® was administered at a dosage of 300 mL per 1000 L of drinking water, as recommended by the producer.

### *Weight parameters of body organs*

The weights of organs (heart, liver, gizzard, and chest muscle), where given, were all calculated by equation = (organ weight /body weight) × 100. During the trial, the body weight of the quails in all groups was calculated in gram according to the age of each group shown in the experimental design.

### *Blood parameters*

Blood samples were collected during slaughter after keeping a half of the quails for the next period. The sample was divided into two parts as follows:

1. Serum samples were separated by blood centrifugation for 15 min at 3000 rpm and reserved at −26ºC until estimation (Nguyen et al., 2014).
2. Blood samples with an anticoagulant were used for complete blood count (RBCs, WBCs) using Natt and Herrick solution and for counting differential leukocyte (DLC)using Wright stain.

### *Follicle parameters*

Follicles were extracted from the body and counted.

### *Percentage of egg production*

Egg production was recorded daily and represented as the percentage of egg production of a hen per day and weekly during the experiment. It has been reported that egg production ceases during the entire forced molting period in all groups (Deek and Al-Harthi, 2004).

% Egg production = (Number of eggs produced per week per number of housed hens) × 100 (Deek and Al-Harthi, 2004).

### *Statistical analysis of the data*

Data of body weight, reproductive organs, ovarian follicles, and blood parameters were collected and compared between control pre-molting, molting, and post-molting birds using descriptive ANOVA analyses with the Duncan multiple range test used at the probability level ($P$ □ 0.05). SPSS version 19.0 and MS Office Excel 2007 statistical tools were used. Data are presented as means ± SD with a significance level of $P < 0.05$ (Steel, 1997).

### **Results**

At the beginning of the experiment, the mean body weight was 225 g with no significant body weight variances between the control and oregostim groups for each period in the pre-molting and the molting period, while in the post-molting period there was a significant variance between group E and group F. The oregostim group showed a restoration and a slight increase in body weight (F) as presented in Table 1. Otherwise, the results showed a significant difference between the periods. There was a decrease in body weight in group C and group D compared with the control group A and a significant increase in group E and group F compared with group B and group C, without a significant variation in the weight of other body organs (heart, muscle, gizzard and liver). There was a significant decline in ovary weight in group C and group D at the molting period and a significant increase in group E and group F compared with other groups. The birds which recovered from molting restored their weight.

Table 2 demonstrates insignificant differences in the pre-molting period in group A and group B in the numbers of red blood cells, white blood cells and differential counts of leukocytes. The analysis of variance indicated that there was a significant decrease in the molting period in group C and group D in red blood cells and white blood cells, respectively, compared with group A and group B, while there were no significant differences in other blood values, but a significant increase was observed in group E and group F in the post-molting period compared with other groups.

Table 3 demonstrates a significant decrease in the percentage of egg production and the numbers of large follicles on the ovary at the molting period compared with groups at the pre-molting period, while it is

*Table 1.* Efficiency of oregostim on body and organ weight in laying Japanese quails in pre-molting, molting and post-molting periods

| Groups | Mean ± Standard Error | | | | | |
|---|---|---|---|---|---|---|
| | Body weight (g) | Heart weight (gm /100 g.bw) | Gizzard weight (gm /100 g.bw) | Muscle weight (gm /100 g.bw) | Ovary weight (gm /100 g.bw) | Liver weight (gm /100 g.bw) |
| Pre-molting period Control (A) | 225.0 ± 2.0 b | 0.846 ± 0.163 ab | 1.92 ± 0.114 a | 25.67 ± 0.65 a | 2.808 ± 0.184 ab | 2.896 ± 0.238 ab |
| Pre-molting period Oregostim (B) | 219.4 ± 5.9 bc | 0.742 ± 0.120 ab | 1.73 ± 0.150 a | 23.90 ± 0.72 a | 3.072 ± 0.192 a | 2.842 ± 0.280 ab |
| Molting period Control (C) | 192.2 ± 4.9 d | 0.682 ± 0.017 ab | 1.66 ± 0.396 a | 22.56 ± 0.18 ab | 1.204 ± 0.041 c | 3.154 ± 0.142 a |
| Molting period Oregostim (D) | 196.6 ± 3.8 d | 0.730 ± 0.016 ab | 2.18 ± 0.089 a | 25.32 ± 0.71 a | 2.126 ± 0.150 c | 3.350 ± 0.118 a |
| Post-molting period Control (E) | 200.0 ± 10.5 c | 0.952 ± 0.040 a | 2.20 ± 0.085 a | 24.79 ± 0.13 a | 3.062 ± 0.211 a | 2.556 ± 0.167 ab |
| Post-molting period Oregostim (F) | 227.6 ± 10.1 a | 0.878 ± 0.078 a | 1.81 ± 0.132 a | 25.55 ± 0.71 a | 3.110 ± 0.147 a | 3.040 ± 0.092 ab |

The letters a, b, c and d indicate the statistically significant differences between groups; $P < 0.05$.

*Table 2.* Efficiency of oregostim on blood parameters of laying Japanese quails in pre-molting, molting and post-molting periods

| Groups | | Mean ± Standard Error | | | | | |
|---|---|---|---|---|---|---|---|
| | RBC × $10^6$ /mm$^3$ | WBC × $10^3$ / mm$^3$ | Lympho-cytes% | Heterophils % | Basophils % | Eosinophils % | Monocytes % |
| Pre-molting period Control (A) | 1.92 ± 0.58 b | 40196 ± 143 a | 70.20 ± 2.08 a | 18.20 ± 1.43 b | 0.600 ± 0.024 a | 8.40 ± 0.44 a | 9.80 ± 1.39 bc |
| Pre-molting period Oregostim (B) | 2.12 ± 1.49 b | 41796 ± 182 a | 76.00 ± 1.76 a | 10.60 ± 1.68 b | 0.600 ± 0.024 a | 1.00 ± 0.44 ab | 12.00 ± 1.30 abc |
| Molting period Control (C) | 1.60 ± 0.71 c | 33513 ± 192 b | 76.00 ± 1.76 a | 10.60 ± 1.68 ab | 0.600 ± 0.024 a | 1.00 ± 0.67 ab | 10.00 ± 1.50 bc |
| Molting period Oregostim (D) | 1.20 ± 1.01 c | 35022 ± 161 b | 71.00 ± 3.56 a | 38.20 ± 2.90 ab | 1.200 ± 0.037 a | 2.60 ± 0.37 ab | 17.00 ± 1.72 ab |
| Post-molting period Control (E) | 3.0002 ± 1.93 a | 42490 ± 247 c | 75.00 ± 3.30 a | 13.00 ± 1.75 ab | 0.400 ± 0.024 a | 0.80 ± 0.80 ab | 10.80 ± 1.59 abc |
| Post-molting period Oregostim (F) | 3.055 ± 1.80 a | 43630 ± 134 a | 79.60 ± 3.55 a | 39.20 ± 1.31 ab | 1.200 ± 0.100 a | 2.20 ± 0.34 ab | 17.80 ± 1.16 ab |

The letters a, b, c and d indicate the statistically significant differences between groups; $P < 0.05$.

demonstrated that there was a significant increase in the percentage of egg production and the numbers of large follicles in the groups at the post-molting period compared with the groups at the molting period.

The current study indicated that the ovarian large follicles during the post-molting period were somewhat bigger than the control at the pre-molting period. However, the oviducts and egg developing follicles appeared comparatively healthier and bigger in post-molted hens (Fig 1). Different sizes of eggs for each period are demonstrated in Fig. 2. Eggs of molted hens are small and have a soft shell compared with the control, while post molt hens had large size eggs, and oregano had a good effect on the

*Table 3.* Efficiency of oregostim on reproductive parameters of laying Japanese quails in pre-molting, molting and post-molting period

| Groups | Mean ± Standard Error | | |
|---|---|---|---|
| | % of egg production | Number of small follicles | Number of large follicles |
| Pre-molting period Control (A) | 52.44 ± 3.1 a | 4.41 ± 0.24 a | 25.8 ± 0.65 a |
| Pre-molting period Oregostim (B) | 56.33 ± 3.22 a | 4.83 ± 0.20 a | 26.2 ± 0.93 a |
| Molting period Control (C) | 31.55 ± 1.44 b | 4.0 ± 0.44 a | 16.2 ± 0.01 b |
| Molting period Oregostim (D) | 32.43 ± 2.12 b | 4.6 ± 0.24 a | 21.6 ± 0.50 b |
| Post-molting period Control (E) | 60.43 ± 2.54 a | 0.51 ± 0.65 a | 30.2 ± 0.33 a |
| Post-molting period Oregostim (F) | 70.65 ± 3.11 a | 0.35 ± 0.66 a | 33.2 ± 0.24 a |

The letters a, b, c and d indicate the statistically significant differences between groups; $P < 0.05$.

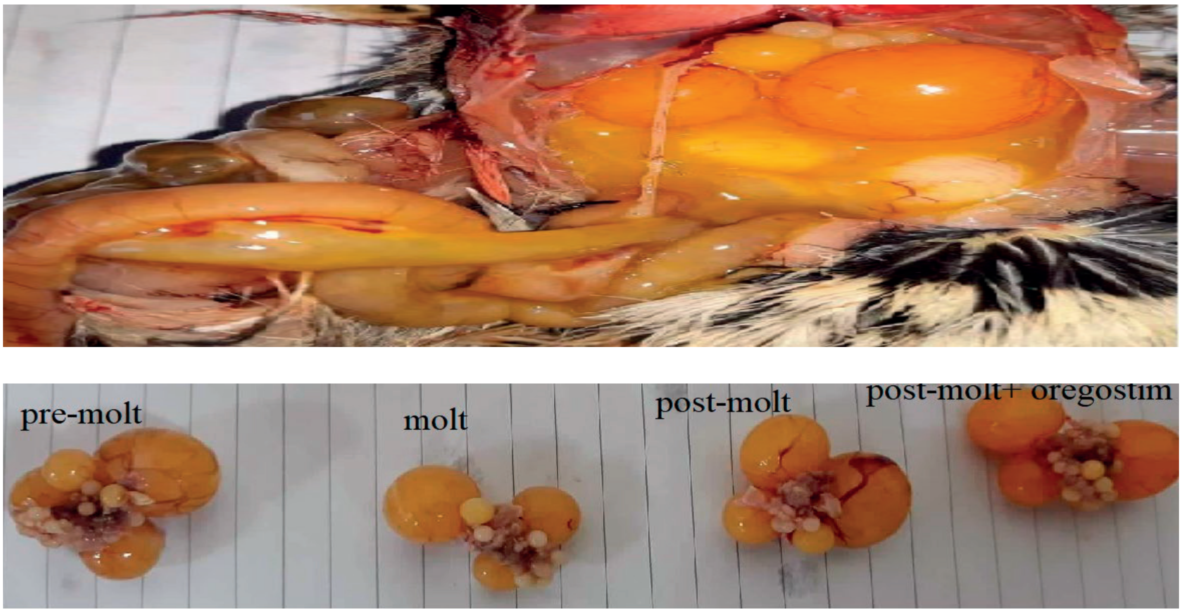


*Fig. 1.* Follicles during the molting periods.

size and shell quality compared with other groups. Fig. 3 shows the appearance of feather falling in molted hens, especially in the neck region, back and thighs. The present study showed that erythrocytes at the pre-molting period were smaller in size and round in shape. During molting, erythrocytes were larger, became more elongated and decreased in numbers, while they increased in their counts and were restored to a normal shape and size at the post-molting period (Fig. 4).

## Discussion

The results of this study demonstrate that induced molt causes a significant decrease in the body weight and ovary weight at the molting period 20$^{th}$ to 22$^{nd}$. This may be due to the stressful effect of induced molt on hens fed a restricted diet because of the lack of minerals, proteins and other supplemented substances in the diet. Otherwise, the supplementation of oregostim in drinking water in molted hens failed to restore the hen's ovary and body weight to normal values during the molting period 20$^{th}$ to 22$^{nd}$, which may be because molt is highly stressful for hens, causing loss of body weight, stagnation of organs of the reproductive tract, cessation of laying eggs, and feather loss (Lee, 1982). Other studies have summarized that the yellow corn feed intake in quails with low protein properties has a reduction effect on body weight and other body organs (Maty et al., 2020). In our study, there was a significant decline in the weight of ovary and large follicles numbers in molted hens with and without oregostim while the weight

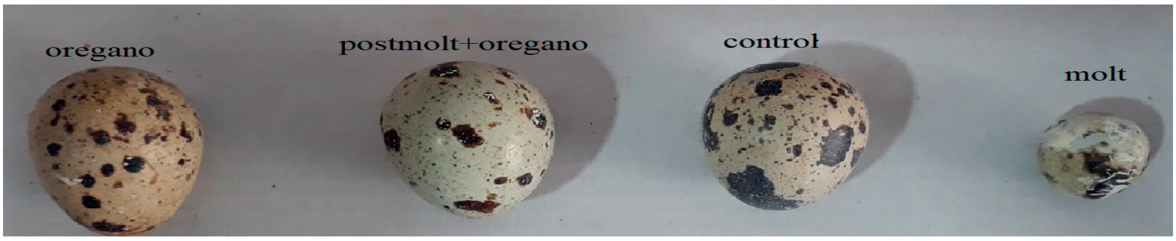


*Fig. 2.* Sizes of eggs for each period.

*Fig. 3.* The appearance of feather falling in molted hens, especially in the neck region, back and thighs.


*Fig. 4.* Erythrocytes during the molting periods.

gain happened in both groups at the post-molting period compared with other groups. This indicates the positive effect of oregostim at the post-molting period 23$^{th}$ to 37$^{th}$ compared with groups without oregostim supplementation at the same period, as it caused weight gain of hens at the post-molting period. Studies have reported that oregostim promotes animal feed intake and enhances and restores the gut lining to improve the animal's health, by renewing the absorbent gut surface (Maty et al., 2020). Oregostim has been reported to cause stimulation of appetite which increases feed consumption (Wen, 2008). Oregostim has an active substance such as thymol, which activates the digestive system and feed intake (Palmer, 1972), increasing the generation of digestive enzymes and improving digestion by promoting liver activities, to stimulate feed intake (Ciftci et al., 2005). This may be the main reason of body weight gain during the post-molting period with oregostim.

Blood parameters of hens are important in acquiring necessary information on the immune status of animals (Brake, 1979). Haematological changes are used to regulate the body status and evaluate the effect of environmental and nutritional stresses (Kohn and Allen, 1955). In our study, we noticed that there were different sizes and shapes of erythrocytes. Erythrocytes at the pre-molting period were smaller and had a round shape. During molting, erythrocytes changed their size and shape. They were bigger, more elongated and decreased in numbers, while they increased in their counts and were restored to normal shape and size at the post-molting period. The difference in the size and forms of red blood cells during molt replicate the definite physiological needs of the organism. The change in the size of red blood cells is the key for perfusion of the tissue, specifically oxygen distribution (Haas and Janiga, 2020). Metabolism increases during molt, so the structure of an erythrocyte affects its morphology, properties and consequently contributes to the ability for gas transfer and blood (Kuenzel, 2003). Changes in blood parameters at the molting period, a significant decrease in both red blood cells counts and white blood cells counts compared with control groups while they get restored to normal values at the post molting period suggest that oregano does not have adverse effects on haematological limits. The various changes in haematological and blood parameters obstruct the birds' ability several systemic functions (Arora and Vatsalya, 2011). Molting modifies hormonal levels, haematological values and blood chemicals (Groscolas and Robin, 2001). A number of theories have explained this drop-in haematocrit during the molt (Robin et al.,1988). Blood parameters might be decreased because of a decline in erythropoiesis secondary to nutritious and energetic imbalance involving some degree of fasting, potentially leading to iron deficiency (Cherel et al., 1988). Previous studies have shown that molting is associated with low concentrations of sex steroid hormones and a severe increase and subsequent decrease of thyroid hormones. These hormonal changes may impact haematological standards (Graw and Kern, 1985).

Another possible explanation for the decrease in haematocrit is hem dilution, an increase in plasma volume as an osmotic adjustment in response to the extensive vascularization of growing feathers without an accompanying increase in erythrocyte population or size (Jouraw et al., 2005). Molting decreases the number of follicles in ovaries. Fasting has been related to autophagy and apoptosis in granulosa cells (Berg and Bearse, 1947). Thus, the causes of a decline in the numbers of ovarian large follicles may be due to a follicle atresia caused by molt, and the body can resorb it to supply energy (Berg and Bearse, 1947). This may disturb the percentage of egg production in molted laying hens which leads to a decrease in the numbers of eggs at the molting period from 20$^{th}$ to 22$^{nd}$. The amount of eggs produced by laying hens increases with age, while the level of reactive oxygen species (ROS) in the ovary and the activity of antioxidant enzymes declines, resulting in an increase in follicle atresia and a decline in laying ability (Oguike, 2005). Consequently, molt causes deterioration of large yellow ovarian follicles and a decrease in the weight of an oviduct, leading to a sharp decrease in egg production (Chowdhury and Yoshimura, 2003). Less blood vessels and a decrease in the expression of FSH and FSH receptors appear in follicles (Bölükbaşı and Kaynar, 2007). In the current study, the restricted diet affected the egg production because hens needed high protein and a sufficient amount of calcium to produce eggs. On the other hand, the water supply with oregostim had no positive effect at the molting period on egg production. Our findings indicate that egg production in laying hens significantly increased at the post-molting period and restored to a normal value compared with control groups at the pre-molting period. Other studies have reported that adding thyme oil and mixed essential oils to the diet increased the total quantity of eggs produced (Han et al., 2022) and supplementing the diet with 100 mg/kg of oreganum considerably enhanced egg production, average egg weight, and food conversion rate (Wang et al., 2021). Plant extracts and spices, individually or in combination, can improve animal performance and health (Jiang et al., 2013; Yang, 2021).

The conclusion from our finding is that oregostim does not have adverse effects on the molting period. It may, therefore, be that the use of a dose of 3 mL /10 L of water is not enough for molted hens. However, it is reported that it has a positive effect at the post-molting period compared with the molting period by using restricted diet. Therefore, the normal diet supplemented with oregostim can be considered a better molting technique.

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