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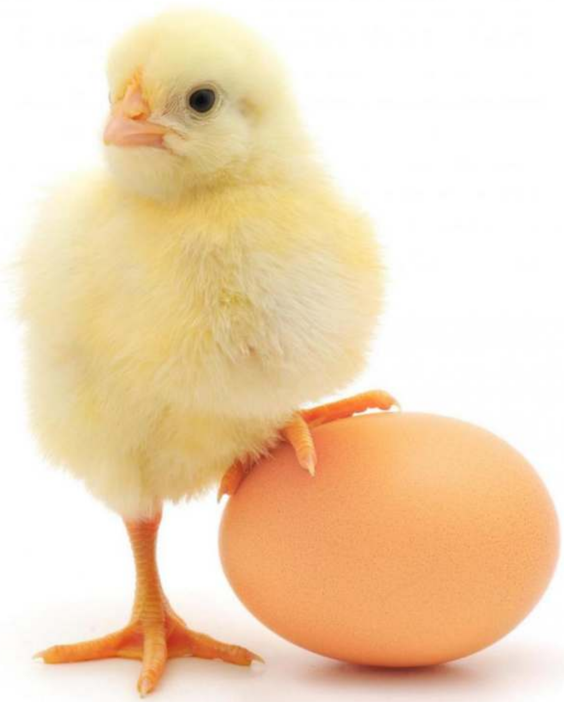

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Deleterious Effects of Molting on the Morpho-physiology of Japanese Quail Layers (*Coturnix japonica*)

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Abstract: Molting is a natural physiological phenomenon involving the periodic replacement of old feathers with new ones in the avian species. During mid-November an extensive loss of feathers in Japanese quail was observed in our breeding colony. The cause of molting could not be established, however, lower ambient temperatures may have played a major role and the decrease in day length could not be ruled out as a contributing factor. This study was conducted to correlate some aspects of the molting process using various physiological and morphometric parameters. Forty healthy 125-days old layers, hatch-mates, of approximately similar body weights (130.0±3.9 g) and in peak production were used for cohort evaluation of the molting process. Most of the birds lost feathers extensively from the cervical, thorax and dorsum areas, while some did not molt and continued laying eggs as usual, serving as a premolting control group. The molting birds drastically lost body weight weighing on average 117.5 g compared to 130.0 g in the control group and ceased egg production completely. There was a significant increase in blood glucose (293.03 mg/dL vs. 222.11 mg/dL), an increase in PCV values (47.14% vs. 41.43%) and a decrease in total plasma proteins (3.5 g/dl vs. 5.56 g/dl) and oviducts (1.55 g vs. 5.78 g, a decrease of 73.2%). Ovarian follicles underwent atresia and resorption. Birds that recovered from the molt resumed egg production and regained their body weights showing similar morpho-physiological measures of the control values, which changed during the molting phase. Scientists working with Japanese quail should be fully aware of the physiology of the molting process and its impact on on-going studies involving growth, physiology, endocrinology, nutrition, reproduction and toxicology.

Key words: Body weight, Japanese quail, molting, reproductive organs

INTRODUCTION

Molting is a natural occurring process in birds; however, the triggering mechanism remains unclear. Various contributing factors known include: shorter day length, low environmental temperature, environmental disturbances, change in feed, shifting of birds to a new location and feed and water restrictions. Artificially-induced molting (forced-molting) is purely done for economic reasons by commercial poultry breeders to extend the reproductive life of the pullets into the second laying cycle without replacing them. It is well recognized that the pullets have to be given some rest from egg production for a varying period of time before resuming the second cycle of egg production, thereby improving their performance (Noles, 1966). Avian investigators continue to search for a safe, effective, less stressful and humane force-molting agent (McCowan *et al*., 2006; AVMA, 2010). The physiological basis of molting and the use of various molt-inducing agents have been reviewed by Payne (1972) and lately by El-Gendi *et al.* (2009). Economically, the Japanese quail serves as a model for forced-molting in the poultry industry, whereas, biomedically, the Japanese quail is an excellent animal model for biomedical research (Huss *et al*., 2008; Dieterlen-Lievre, 1997).

Evidence of molting includes interruption in the secretion of gonadotropin and the associated steroid hormone and redirecting the body's metabolic system towards thermoregulation and the replacement of feathers (Brake and Thaxton, 1979a), which in turn, brings about regression of the reproductive organs (oviducts, ovaries and follicles) and the cessation in egg production (Harrison *et al*., 1974). Stressful effects of molting manifests are lowered metabolic energy needs and interrupted hormonal secretions (Payne, 1972). Molting, if occurring during an on-going experiment, could pose to be highly detrimental to the accuracy of the data from studies on growth, reproduction, physiology, pharmacology, endocrinology, egg production and toxicology. Few studies have demonstrated or established the extent of alterations that can take place during the molting process. Accordingly, the objectives of this study are: 1. Characterize the morphometric and physio-chemical responses in Japanese quail during the molting process and 2. Evaluate and standardize the potential effects of molting on the Japanese quail for biomedical research.

MATERIALS AND METHODS

The birds used in this study were fully acclimated to the habitat and were in peak egg production status prior to

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molting. They were housed in a temperature controlled room at ~73° F with a 16 L: 8 D lighting system with access to feed and water *ab libitum* (Arora, 1979). Routine time-bound evaluations are being conducted with Japanese quail to establish and validate correlational growth patterns, blood chemistry, egg production and reproductive parameters year round. In late fall (mid-November), several birds suddenly went into a heavy molt while others did not molt and continued to lay eggs as usual. This type of event had never been seen before in our facility. A total of forty 125-days old Japanese quail birds, hatch-mates, with lastly recorded similar body weights of 130.0±3.9 g were used in this study. The birds were divided into two groups: Group I: Heavy Molting (n = 24) and Group II: Non-molting (n = 16), which continued to lay eggs as usual and served as premolting layers (controls). Molting birds were evaluated during the course of study time line during the molting and postmolting periods. Birds were weighed individually at the designated time-points to the nearest 0.1 g and approximately 0.5 ml of blood was collected from the brachial vein using a tuberculin syringe and a 1-inch 25-gauge needle; the blood was then immediately transferred to EDTA-coated vials. At each observational event, the body weight, Packed Cell Volume (PCV), blood glucose, plasma proteins, specific density and refraction of plasma were recorded for comparison. PCV was determined with microhematocrit tubes using the Unico C-MH30 appliance and plasma was extracted using micropipettes. Blood glucose was determined with glucometer (Elite XL) immediately after blood collection, whereas, plasma glucose was determined after centrifugation of the hematocrit tubes. Total plasma proteins, refractory index and specific gravity were recorded simultaneously using clinical refractometer (T2-Ne Atago Co). Following blood collection, eight birds each from Group I and Group II were euthanized with CO_2 gas in separate small animal anesthesia chambers. Their oviducts were excised and weighed to the nearest 0.1 mg and the ovarian follicles were measured to the nearest millimeter (mm). During the first observation event, the Group I molting birds weighed approximately 10% less than the control birds. Upon full recovery from molt, the remaining birds (n = 8) had gained weight, developed new feathers, resumed egg production and recovered blood values, however, there was a considerable individual variation in the recovery process. During the postmolting phase (approximately 75 days from the start of experiment), the remaining birds from the molting group (n = 8) and a similar number from control group (n = 8) were euthanized and evaluated using procedures similar to those used in the earlier batch. The remaining Group II control birds were kept for observation purposes only throughout the study time line. Data collected on body weight, reproductive organs, ovarian follicles and blood constituents were analyzed for comparison among control (premolting), molting and postmolting birds using descriptive, MANOVA analyses using SPSS version 19.0 and MS Office Excel 2007 statistical tools. Data is presented as means±S.D. and minimum-maximum ranges at the significance level of 0.05 and higher. No data was recorded on feed and water consumption, egg production and behavior since these factors were not included in the objectives of this study. Some data were excluded due to extreme outlying values and deaths.

RESULTS

Observations were made on the status of various morphological and laboratory parameters such as body weight, reproductive organs, PCV, blood plasma proteins, specific gravity, refractive index, glucose levels during premolting (control), molting and postmolting periods (Table 1). Group I birds shed feathers heavily around the cervical, thoracic and dorsum areas and had ceased egg laying completely. Heavy molting continued for 16-18 days, gradually transitioning into a stage consisting of the growth of pin-like feathers in the molted areas, a phase which continued for three to four weeks towards recovery (postmolting phase; Fig. 3). The remaining eight birds in Group II (non-molting) kept for observation, continued laying eggs as usual and did not show any alterations in both blood and morphological evaluations. The molting birds from Group I lost body weight reaching the lowest mean value of 117.5±2.96 g vs. 130.0±3.9 g in controls, a decrease of 9.6%. The postmolting birds weighed 131.5±4.08 g, approximately 1.2% more than the premolting readings.

Table 1: Means and ranges (minimum-maximum) of various morpho-physiological parameters during premolting, molting and postmolting periods

Parameters	Premolting laying period	Molting period	Postmolting laying period
Body weight (g)	130.00 (124.4-133.2)	117.50 (100.0-120.2)	131.50 (122.8-135.8)
Blood glucose (mg/dL)	222.11 (200-264)	293.03 (247-381)	261.43 (214-319)
Plasma glucose (mg/dL)	315.20 (279-382)	352.55 (294-437)	324.63 (286-352)
PCV (%)	41.43 (36.0-48.5)	47.14 (40.0-51.5)	42.50 (39-46)
Serum protein (g/dL)	5.56 (3.6-7.4)	3.50 (2.4-6.2)	5.89 (4-8)
Refraction (RIS)	1.35 (1.34-1.35)	1.34 (1.33-1.35)	1.35 (1.34-1.35)
Specific gravity	1.04 (1.03-1.05)	1.03 (1.02-1.04)	1.04 (1.03-1.05)
Ovary weight with follicles (g)	8.50 (5.00-10.20)	1.40 (0.05-2.12)	4.80 (2.27-5.34)
Oviduct weight (g)	5.78 (3.2-8.57)	1.55 (0.08-3.41)	4.75 (1.02-7.37)

Molting birds had higher blood glucose levels of 293.03±16.28 compared to premolting birds at 222.11±24.42 mg/dL, an increase of 31.93% and an increase of 12.09% compared to the postmolting birds, with levels of 261.43±37.81 mg/dL. The blood glucose level was 5.04% higher in postmolting birds compared to premolting birds. There was a similar trend in plasma glucose values. Molting birds had PCV levels of 47.14±0.64, an increase of 12.1% compared to pre-molting values of 41.43±3.7 and a 9.8% increase compared with postmolting values of 42.50±2.62%. Postmolting birds had a higher PCV value of 1.13% over the premolting values. Clinical refractometer readings reflected total plasma proteins, specific gravity and refraction values during pre-molting, molting and post-molting phases. During molting, mean values of total plasma proteins was reduced by 37.05% from pre-molting values of 5.56±0.3 g/dL to 3.50±0.63 g/dL by 10.92% from a post-molting level of 5.89±1.38 g/dL. Postmolting birds showed a slightly higher plasma protein level (5.9%) compared to pre-molting concentration. There was a non-significant decline in the Refractive Index (RIS) reading during the molting period when compared to premolting and postmolting values. Specific gravity during the molting phase was 1.03±0.02, a slight decrease from premolting and postmolting values of 1.04±0.01. The correlational analysis showed a correlation factor of r = 0.946 ($p<0.01$) in the variations of parametric data between premolting and molting periods and r = 0.988 ($p<0.01$) between molting and postmolting data. There was no significant change in specific gravity values between the premolting and postmolting birds. Detailed data on these parameters is provided in Table 1 and data on percentage deviations is listed in Fig. 2. The ovaries had regressed drastically and were devoid of follicles containing yellow yolk and there was evidence of follicular atresia and resorption of follicles. Mean weight of the ovary was drastically reduced to 1.4 g compared with a pre-molting phase of 8.5 g, a decrease of 83.7% and a decrease of 70.8% from the post-molting value of 4.8±1.09 g.

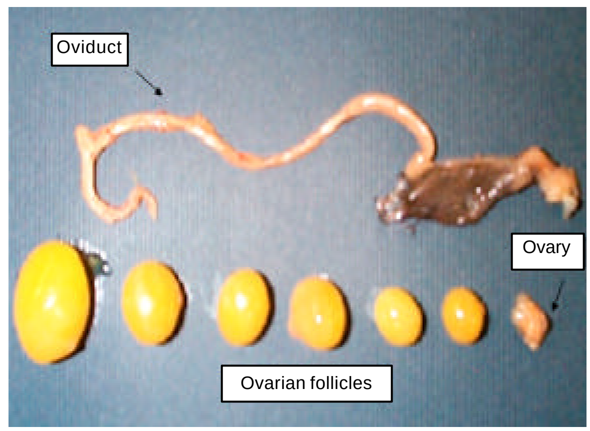
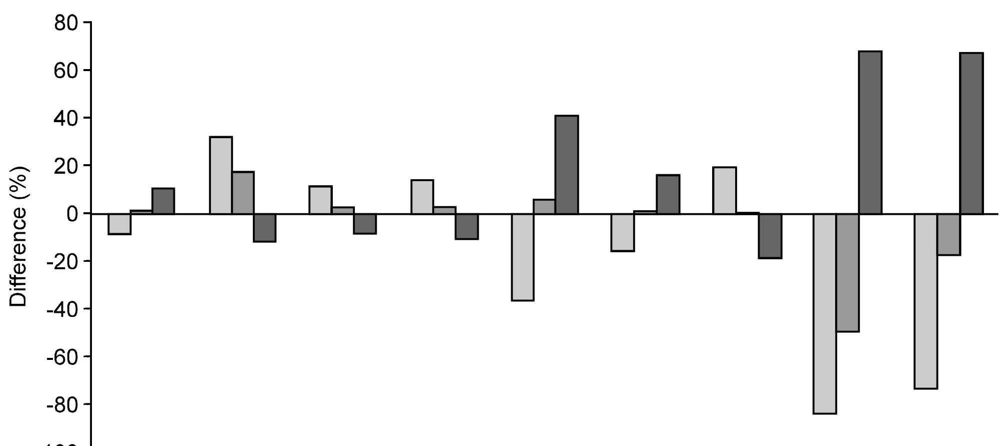


Fig. 1: Oviduct, ovary and graded ovarian follicles in a postmolting bird



	Body weight	Blood glucose	Plasma glucose	PCV	Serum protein	RIS	Specific density	Ovary weight	Oviduct weight
Molt and premolt	-9.63	31.93	11.85	13.80	-37.11	-15.87	19.48	-83.68	-73.12
Postmolt and premolt	1.19	17.70	2.99	2.60	5.89	0.14	0.08	-49.51	-17.67
Postmolt and molt	10.69	-12.09	-8.60	-10.92	40.61	15.98	-19.37	67.68	67.35

Fig. 2: Percentage differences in various parameters during premolting, molting and postmolting periods

Fig. 3: Growing new feathers in a postmolting bird

The recovery of ovarian weight was delayed during the post-molting phase, 43.5% lower than control values. Mean oviduct weight was reduced to 1.55 g, a decrease of 73.2% during molting compared to the pre-molting value of 5.78 g and by 67.4% of the postmolting value of 4.73 g. The recovery process for the oviduct and the ovary were highly correlated with $p<0.05$ and were independent of recovery rates of physiological parameters. Mean oviduct weight during post-molting was 17.8% lower than pre-molting values. During molting, the larger ovarian follicles were atrophied and appeared ruptured and contained blood-tinged greenish fluid. The ovarian follicles during post molting were somewhat bigger than the controls. The mean values for the first five graded follicles were 16.2, 12.0, 8.1, 6.0 and 4.0 mm compared to 15.8, 10.9, 6.7, 4.2 and 4.1 mm in the control group ($p>0.05$). During the molting phase, the females were hard to distinguish from males, because the neck and breast were completely devoid of plumage. The birds which recovered from molt gained weight, resumed egg production and returned to normal glucose, PCV and proteins values, whereas, the mean weights of the oviduct and ovary were somewhat slower in recovering. However, the oviducts and developing follicles appeared comparatively healthier as shown in Fig. 1.

DISCUSSION

Molting caused a temporary pause in egg production and the regression of reproductive organs. The triggering mechanisms remain largely unclear. However, various known contributing factors are shorter photoperiod, low environment temperature, feed and water deprivation, shifting of birds, quality of diet, migration and breeding season. (Palmer, 1972). What triggered molting in our breeding colony during mid-November (onset of a colder weather) is unclear. We assumed that the low ambient temperature during those days initiated molt, however, the role of the shorter photoperiod could not be ruled out. Molting as a result of lower temperature (Thompson and Boag, 1976) and shorter photoperiod (Hall *et al*., 1993) have been reported. The intensity of molt was assessed by the number of shed feathers found on the floor of the cages. Molting is very stressful on the birds accounting for a loss in body weight, involution of reproductive organs, cessation of egg production, thermoregulatory setback owing to loss of feathers (Sekimoto *et al*., 1987) and changes in various hematological and blood chemistry parameters which hamper the birds' ability to carry out various systemic functions (Brake and Thaxton, 1979b; Brake *et al*., 1979c).

Molting also alters hormonal levels, hematological values and blood chemicals. These include elevations of blood glucose, PCV and a decrease in total plasma proteins as presented in our study. These findings corroborate the earlier reports of Parek and Sulman (1945) and Brake and Thaxton (1979a,b) from their studies on forced-molted chickens resulting in a loss in body weight, regression of reproductive organs, an increase in PCV and hemoglobin, a decrease in plasma calcium and total proteins, a decrease in the size of liver and an increase in the size of the right adrenal gland.

The birds that recovered from the molt regained body weight and resumed egg production. However, there was a wide individual variation among the birds during the postmolting recovery phase. Mean weights of ovaries and oviducts were smaller during the postmolting phase though they appeared comparatively healthier compared to the non-molting control group. Similarly, the size of the ovarian follicles was somewhat bigger than corresponding controls. Blood and plasma glucose, PCV and plasma protein values, which altered during molting, recovered to normal values quicker than the ovaries and oviducts.

In conclusion, it is safe to say that molting, if it occurs during an on-going experiment, may result in erroneous conclusions about various morpho-physiological parameters such as body weight, reproductive organs, hormones, blood parameters and metabolism.

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