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Effect of egg size, age of hen and storage period on fertility, hatchability, embryo mortality and chick malformations in eggs of japanese quail (*Coturnix coturnix japonica*)

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Abstract: This study was conducted to investigate the effect of egg size, age of hen and storage period of eggs before incubation on fertility, hatchability, embryo mortality and chick malformations in Japanese quail (*Coturnix coturnix japonica*). The study was carried out in quail farm located at Tanah Merah, Kelantan. Totally 1441 eggs of quail were set into the incubator by applying 9 treatments groups: 3 groups for egg size (6-7 g, 8-9 g and 10-11 g), 3 groups for hen age (≥ 3 - <5 month, ≥5 - <8 month and ≥8 - <10 month) and 3 groups for egg storage period (≥ 4 - ≤6 days, ≥7 - ≤9 days and ≥10 - ≤14 days). Records on fertility, hatchability, embryo mortality and chick malformations were taken from each hatch. Analysis of variance (ANOVA) revealed that the egg size had highly significant (P< 0.01) effect on hatchability and embryo mortality in quails and was not found to be significant (P>0.05) on fertility and chick malformations. The age of hen had insignificant (P>0.05) effect on every aspect except fertility. Storage period had highly significant (P<0.01) effect on fertility, hatchability and embryo mortality rate. Results from this study concluded that heavy egg weight (10-11 g), middle aged hen (≥5 - <8 month) and shortest storage period (≥4 - <6 days) gave the best hatching performances.
Key words: *Japanese Quail, fertility, hatchability, embryo mortality, chick malformations, storage period*

I. Introduction

The poultry sector is the largest of all among the livestock industries in Malaysia. The poultry production is practiced at levels ranging from subsistence to large scale commercial operations. The commercial operations depend on the hatcheries for the supply of day old chicks while the subsistence farmers hatch their chicks by natural incubation (King'ori, 2011). The uninterrupted supply of quality day old chicks is mainly necessary for the success of production chain. For this reason, the quail breeder needs to maintain the high fertility, high hatchability and also good quality of chicks. Compared to chicken hatcheries, quail hatcheries are less equipped and the owners do not practice strictly the management schedule resulting in poor fertility, hatchability and large proportion of malformed chicks. There are many causes that contribute in poor fertility, hatchability of eggs and poor quality of chicks. Some of them are improper temperature and humidity, poor sanitation, the number of male and female ratio, longer storage period, improper setting eggs and others. Therefore, the present experiment was conducted to examine the effect of egg size, hen age and storage period on fertility, hatchability, embryo mortality and chick malformations in newly hatched quail chick.

II. Materials and Methods

This study was carried out at a layer quail farm, Lot 1777, Jalan Lubok, Tanah Merah, Kelantan, Malaysia. The research aimed at identifying and quantifying the effect of egg size, hen age and storage period of quail eggs before incubation on fertility, hatchability, embryo mortality and chick malformations in newly hatched chicks. The whole research was accomplished in 3 separate experiments viz Experiment I (Effect of egg size), Experiment II (Effect of hen age) and Experiment III (Effect of storage period of eggs) to examine the effect of different factors on fertility, hatchability, embryo mortality and chick malformations. For the experiment I, the quail eggs were collected from the layer quail flock consisted of 50 quails with 1:3 cock-hen ratio. The collected eggs were weighed individually by using the digital scale with the sensitivity of 1.0 g and were grouped based on their weight as light (6.0-7.0 g), medium (8.0-9.0 g) and heavy (10.0-11.0 g) weight groups. For the experiment II, The quail eggs were collected from 3 separate flocks varied according to the age of hens (≥3 - <5 month, ≥5 - <8 month and ≥8 - <10 month) and for the experiment III, the quail eggs were collected and divided into three groups and stored at room temperature for 3 different periods (G1: ≥4- ≤6 days, G2: ≥7-≤9 days and G3: ≥10-≤14 days) prior to incubation. All the eggs were set on the tray based on their egg weight groups, hen age groups and storage period groups before placed into the incubator. The eggs were set at 36.0° C for the period from 1until 14 days of incubation and thereafter reduced by 1°C. The temperature and ventilation were automatically adjusted. The eggs were turned around manually once every two hours started from 7.00 am until 9.00 pm per day. The turning of eggs was stopped on 15th day of incubation prior to hatching. The cabinet incubator was used for incubating the eggs. The number of quail eggs set was counted and recorded.

The eggs were candled on 7th day of incubation period in a dark room with the eggs held before a light. The infertile eggs were determined by the appearance of clear interior of the eggs and the fertile eggs were determined by presence of blood vessels. The numbers of infertile eggs were recorded. The quail eggs were transferred from setting tray to hatching tray on 15th day of incubation. The eggs used to be hatched within 17-18th day. The number of newly quail chicks hatched and the eggs that not hatched were counted. Any malformation of the chicks also was examined and recorded. The hatching parameters were calculated using the following formulae:

$$\text{Fertility rate (\%)} = \frac{\text{Number of fertile eggs}}{\text{Total number of eggs set}} \times 100$$

$$\text{Hatchability of fertile eggs (\%)} = \frac{\text{Number of hatched chicks}}{\text{Total number of fertile eggs}} \times 100$$

$$\text{Embryo mortality rate (\%)} = \frac{\text{Number of dead embryos}}{\text{Total number of fertile eggs}} \times 100$$

$$\text{Chick malformations rate (\%)} = \frac{\text{Number of chick malformations}}{\text{Total number of fertile eggs}} \times 100$$

Data on fertility, hatchability, embryo mortality and chick malformations rate were subjected to analysis of variance (ANOVA) to examine whether any difference exists between groups under each experiment. Means in each experiment were separated by Duncan's New Multiple Range Test (DMRT) using SPSS version 16.0 (Yaacob, 2011) in case when ANOVA was significant (Kaps and Lamberson, 2009).

III. Results and Discussion

The results in terms of effects of egg size, hen age and storage period of quail eggs on fertility, hatchability, embryo mortality and chick malformations are described under the following sub-headings

Effect of egg size on fertility rate: ANOVA shows that there was no significant difference ($P > 0.05$) between the effects of egg size on the fertility rate of eggs (Table 1). The fertility of quail eggs in different size group ranged between 84.72% and 90.10%. However, medium sized eggs (8-9 g) manifested insignificantly ($P > 0.05$) highest (90.10 %) percentage of fertile eggs determined by candling on day 7 of incubation.This result was consistent with the research conducted in Turkey by Copur et al., (2010) who noticed that the egg size had no significant effect on the fertility rate in Japanese quail eggs. However, some differences in fertility were noted between the egg size groups in the research conducted in Turkey by Seker*et al.*, (2005). In their experiment, fertility rates found on light (9.50-10.50 g), medium (10.51-11.50 g) and heavy (11.51-12.50 g) eggs were 69.72%, 75.83% and 79.81% respectively indicating a trend that fertility increased as the egg weight increased. Nonetheless, this contradiction might be due to differences in experimental design such as grouping of eggs based on size and number of eggs set. Besides, the probable causes in difference among of fertility rate could be due to age of the parents, male female ratio and many other environmental factors. Brammel *et al.*, (1996) conducted their experiment in United States and found that factors affecting fertility originate from male that include sperm quality traits like sperm motility, sperm metabolism, semen concentration and percentage of abnormal or dead sperm cells. Meanwhile, the fertility factors originating from female include egg quality, behavior and physical factors like prevalence of sperm storage tubules of quails (Siegel, 1965). However, from present findings, it shows that the quail eggs from any of egg size groups (6-7 g, 8-9 g and 10-11 g) could be chosen for incubation since there was insignificant ($P>0.05$) effect on the fertility rates.

Effect of egg size on hatchability rate : ANOVA reveals that the egg size had highly significant ($P<0.01$) effect on hatchability of quail eggs set for hatching. The rate of hatchability based on fertile eggs for largest, medium and smallest sized eggs were observed as 80.95, 60.45 and 66.73percent respectively (Table 1). Duncan's New Multiple Range Test (DMRT) shows that the largest egg size group had significantly ($P<0.05$) highest hatchability (80.95%) followed by other two groups with insignificant difference ($P>0.05$) between the medium (60.45%) and smallest (66.73%) egg size group.These results correspond with result conducted by Sachdev *et al.*, (1985) in India who noted that the hatchability of the fertile eggs was highest in heavy (10.1-11.0 g) eggs. Study in Bangladesh reported by Uddin *et al.*, (1994) indicated that the highest hatchability of the eggs was found in medium (9.10- 10.00 g) egg size group in quail. Another finding related to the egg size showed that hatchability of fertile eggs increased as the egg weight increased. Again the result indicating the highest hatchability of all eggs set was observed in eggs $\geq$ 11.6 g in research done in Turkey (Sarica and Soley, 1995). Besides, the differences among hatchability results may have been resulted from variations in parent factors, feeding conditions, egg storage conditions and related environmental conditions. There might be many factors contributed to the failure of fertile eggs to hatch which included lethal genes, insufficient nutrients in eggs and exposure to conditions that do not meet the needs of the developing embryos (King'ori, 2011).

Effect of egg size on embryo mortality rate : The influence of egg size on embryo mortality rate of eggs set was found to be highly significant (P<0.01) as appeared in ANOVA. Percentage of dead embryos based on fertile eggs varied within the range of 17.86 and 39.55 percent. The highest egg size group showed significantly (P<0.05) lowest embryo mortality rate. However, there was no significant (P>0.05) difference between the medium and lowest egg sized group in the regard (Table 1).This finding is in agreement with the experiment conducted in Turkey by Seker *et al.*, (2005) that the embryo mortality rate of lightest (9.50- 10.50 g) egg size group was higher than of medium and largest egg size groups, indicating that embryonic mortality decreases as the egg weight increases. This situation may be explained as heavy weight eggs have sufficient nutrients and centrally placed yolk to support embryonic growth and development compared to light weight eggs (Seker*et al.*, 2005).

Effect of egg size on chick malformations rate: Malformed chicks were shown to have insignificant (P> 0.05) difference according to the effect of egg size expressed in percentage (Table 1). Numerically, the rate of quail chick malformations based on fertile eggs for smallest, medium and heaviest egg sized group were obtained as 7.72, 6.03 and 4.76 percent respectively.Numerically, the smallest egg size had the highest malformed chicks with the average number of chick abnormality found in wet chicks. In medium egg size, the characteristic of chick abnormality had been found on wet chick, paralyzed chick, and bloody navel. In largest egg size groups, the chick abnormality only was found on wet chick. There are some corrective measures that can be taken to improve quality newly hatched quail chicks such as to follow the recommended temperature settings, increase humidity by water evaporation, proper ventilation, proper egg handling and the eggs must transferred to hatching tray from setting tray in the incubator at its proper time. However, no literature was available on the effect of egg size on the incident of chick malformation during hatching in quails.

Effect of age of hen on fertility rate: ANOVA resulted in highly significant (P< 0.01) difference between the effects of age of hen on fertility rate of eggs (Table 2). Duncan's New Multiple Range Test shows that the largest (P< 0.05) fertility rate among the age of hen groups was found in middle aged hens with an average 98.94 percent, followed by 94.91 percent in oldest age and 90.20 percent in youngest group of hens. The fertility decreased significantly (P>0.05) with the increase in hen's age in this research. Alsobayel (1992) stated that hen age had an influence on fertility of Baladi chicken eggs and there was a general tendency of fertility to decline with the progress of age (Insko*et al.*, 1947). Still, the hens would undergo some physiological changes as age affect their ability to fertilize their egg upon mating.

Effect of age of hen on hatchability rate: The analysis of variance reveals that there was insignificant (P> 0.05) difference between the effects of age of hen on the hatchability rate of eggs set based on fertile eggs. However, the eggs from hens of middle age group had numerically largest (76.96 %) hatchability among the age groups of hen and the oldest age group showed smallest hatchability rate (Table 2). In support, in an experiment conducted in Turkey by Dere *et al.*, (2009) noted that there was no statistically significant (P> 0.05) difference between 71.11 percent and 72.89 percent hatchability values in quail eggs obtained from breeders of 3 and 6 months age. In the same experiment the hatchability rate was found to be reduced due to increasing age higher than 8 months and above. This finding was in agreement with experiment conducted by Dudusola (2013) in Nigeria who noticed that the hatchability of fertile eggs was reduced due to increasing parental age. In his experiment, hatchability of 5.5 month and 9 month of hen age were 96.05 percent and 78.85 percent respectively. The reduction in hatchability due to increase in maternal age as revealed in the study might be due to certain physiological characteristics due to aging apart from egg size, egg quality, different management practices and environment conditions.

Effect of age of hen on embryo mortality rate: ANOVA shows that there was no significant (P> 0.05) difference between the effects of age of hens on embryo mortality rate of eggs (Table 2). Nevertheless, the rate of embryo mortality for youngest, middle and oldest aged groups were numerically observed as 25.60, 23.04 and 29.45 percent respectively. Ritsareva (1991) noted that hatching of first laid quail eggs was not satisfactorily hatched. Petek and Dikmen, (2006) concluded that the reproductive potential in quails held at a high level during 6 to 8 months and thereafter, the fertility rate decreased and embryonic death increased.

Effect of age of hen on chick malformations: ANOVA reveals that the age of hen had insignificant (P> 0.05) effect on malformation of newly hatched quail chicks. However, the eggs from middle aged group have numerically largest (2.69%) chick malformations among the age groups of hens and the oldest aged group showed lowest chick malformations (Table 2).The chick malformations in all hen age groups were observed in wet chicks. Generally, some deformities in newly hatched chicks can be avoided by correcting the incubator settings such as temperature and humidity and possibly by providing balanced flock nutrition (Gauthier and Ludlow, 2013). According to Avian Business Unit (2007) in France there are two different consequences on chick quality which are; too early hatching results in dehydrated chicks when exiting the hatcher and the another one was too late hatching leads to weak and wet chicks with frequent omphalitic problems. However, there was no literature available on the influence of hen age on malformation of newly hatched quail chicks.

Effect of storage period on fertility rate: Eggs were stored at room temperature in three different storage periods and ANOVA showed that there was highly significant ($P< 0.01$) effect of storage period on fertility of eggs set for hatching (Table 3). Fertility of quail eggs according to longest to shortest storage period group ranged between 78.62 to 96.46 percent. Duncan's New Multiple Range (DMRT) test shows that fertility rates linearly and significantly ($P<0.05$) decreased with the length of storage period where highest fertility was noted among the eggs stored lowest period (≥ 4 - ≤ 6 days). Again, significantly ($P<0.05$) lowest fertility was recorded for egg stored longest time (≥ 10 - ≤ 14 days). From the result obtained it shows that the trend of fertility rate may decrease as the storage period increases. This trend can be supported by the findings of Uddin *et al.*, (1994) in Bangladesh who noticed that storage period of eggs before incubation had highly significant ($P<0.01$) effect on fertility rates of eggs set. The highest fertility was obtained from eggs stored for 4 days with an average fertility of 83.60 percent and the fertility rate reduced to 83.24 and 82.49 percent in 7 and 10 days storage period (Uddin *et al.*, 2004). Fertility or infertility of egg is determined before the egg is laid. Apparently there should be no effect of storage period on fertility. Effect of storage period in this experiment may be explained by the degeneration of early embryonic cells during long time storage in room temperature before incubation because egg showing no blood vessel during candling was considered infertile although it might be fertile.

Effect of storage period on hatchability rate: The influence of egg storage period on hatchability rate was found to be highly significant ($P< 0.01$) as appeared in ANOVA. The rate of hatchability for shortest, intermediate and longest storage periods were observed as 70.83, 55.66, and 55.75 percent respectively (Table 3). Duncan's New Multiple Range Test shows that the shortest storage period group had significantly ($P< 0.05$) largest hatchability rate followed by other two groups with insignificant ($P> 0.05$) difference between the intermediate and longest egg storage period. This result was in agreement with the study done by Lacin*et al.*, (2008) in Turkey, who reported that influence of storage period (1-3 days, 6-8 days, 12-14 days) on the hatchability of fertile eggs values (66.5 %, 56.83 % and 18.93 %) was demonstrated to be highly significant ($P< 0.01$) and the extension of the storage period more than 8 days resulted in higher decreased hatchability values of fertile eggs.Besides, the study by Azeem and Azeem (2009) in Egypt reported that the best hatchability percentage was observed for groups of eggs stored for 4 days (76.27 %) as compared with other storage periods 2 days (72.12 %) and 6 days (71.81 %). From this study, it can be concluded that as the storage period increase, the hatchability rate of fertile eggs would be decreases. The results of this study provide evidence that the quail eggs that stored longer than 7 days were detrimental to hatchability.

Effect of storage period on embryo mortality rate: ANOVA indicates that the egg storage period had highly significant ($P< 0.01$) effect on embryo mortality rate of quail eggs set for hatching. Percentage of dead embryo based on fertile eggs varied within the range of 28.51 to 45.96 percent. The shortest egg storage period group showed significant ($P< 0.05$) lowest dead embryos. However, there was insignificant ($P> 0.05$) difference between intermediate and longest egg storage period group in this regard (Table 3). This result was consistent with the experiment carried out in Turkey by Seker *et al.*, (2005) who noticed that there was highly significant ($P< 0.01$) difference between the effect of storage period (≤ 3 days, 4-6 days, 7-9 days, 10-12 days and 13-15 days) on embryo mortality rates. Similarly, the result reported by Lacin *et al.*, (2008) tells that the storage period (1-3 days, 6-8 days and 12-14 days) had highly significant ($P< 0.01$) effect on early death rates and interaction between egg storage period groups was significant ($P< 0.05$). As a result of extended storage period, embryos mortality rates increased because of water loss and albumen degradation during storage. In such a case, the various precautions may be required to be taken starting from the environment in which the eggs are kept to the placement of the quail eggs into incubators. In the present study, the place used for egg storage was exposed to the environmental temperature and humidity and throughout the storage and these factors might contribute embryo mortality rate to elevate.

Effect of storage period on malformation of chicks: Egg storage period was not found to have significant effect ($P>0.05$) on malformation of newly hatched quail chicks (Table 3). Nevertheless, eggs from longest storage period group have numerically largest (2.72%) chick malformations among the storage period groups and the shortest storage period showed the lowest (0.52 %) chick malformations rate. However, it shows that the higher the storage period, the higher in the proportion of malformed chick although insignificantly ($P>0.05$) (Table 3).The chick malformations that found in all the storage period groups were in wet chick. According to Brake (1989), the eggs loose a certain amount of weight due to dehydration during storage and incubation starting from oviposition until hatching, and this weight loss had a major influence on incubation processes and chick quality. However, there was no literature available for the study on effect of storage period of quail eggs prior to incubation on the malformation of newly hatched quail chicks.

IV. Conclusion:

With the exception of hatchability and embryo mortality, there was insignificant (P> 0.05) effect between egg sizes on fertility and chick malformation in quails. With respect to hatchability, the heavy eggs (10-11g) gave the best result and at the same time, reduced the incidence of dead embryos in quail eggs compared to light and medium sized eggs. So, the heavy eggs could be preferred by the quail breeder to increase the hatchability rate of newly hatched chicks. Nonetheless, the economic cost of hatchability has to be considered as well as favourable outcome for the breeder. Besides, the breeder can produce the uniform size of chicks at the market age if the eggs can be sorted by their weight. The fertility decreased significantly as the progress of hen age in this study. Eggs from the middle aged hen (≥5 - <8 month) showed the better fertility rates among the hen age groups (≥3 - <5 month and ≥8 - <10 month). On the other hand, the hatchability, embryo mortality and chick malformations were not found to be affected by age of hen. In case of storage period of eggs, with exception of chick malformations, there was significant effect on fertility, hatchability and embryo mortality in quail eggs. From this study, it can be recommended that the optimum storage length should be ≤6 days because the trend of fertility and hatchability would be decreased as the storage length extends. However, it warrants pursuing further research on the role of these factors on fertility, hatchability, embryo mortality and chick malformations of quail chicks in artificial incubation with large number of samples.

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Table 1: The effect of egg size on fertility, hatchability, embryo mortality and chick malformation in newly hatched chicks

Egg size (g)	No. of eggs set	Mean Fertility (%) ($\bar{X}$ ± SE)	No. of fertile eggs set	*Mean Hatchability (%) ($\bar{X}$ ± SE)	*Mean Embryo mortality (%) ($\bar{X}$ ± SE)	*Mean Chick malformations (%) ($\bar{X}$ ± SE)
6-7	46	84.72 ± 2.37	39	66.73^b ± 1.43	33.27^b ± 1.43	7.72 ± 4.46
8-9	202	90.10 ± 0.47	182	60.45^b ± 2.44	39.55^b ± 2.44	6.03 ± 3.48
10-11	49	87.50 ± 1.71	42	80.95^a ± 2.38	17.86^a ± 3.57	4.76 ± 2.75
F-value		3.09 NS		10.77**	19.58**	0.85 NS

NS = not significant (P> 0.05) ** P <0.01*Means with different superscripts differ significantly (P<0.05)

Table 2: The effect of hen age on fertility, hatchability, embryo mortality and chick malformation in newly hatched chicks

Age of hen (month)	No. of eggs set	*Mean Fertility (%) ($\bar{X}$ ± SE)	No. of fertile eggs set	Mean Hatchability (%) ($\bar{X}$ ± SE)	Mean Embryo mortality (%) ($\bar{X}$ ± SE)	Mean Chick malformations (%) ($\bar{X}$ ± SE)
≥3 - <5	174	90.20^a ± 1.14	157	74.40 ± 4.97	25.60 ± 4.97	1.87 ± 1.09
≥5 - <8	188	98.94^b ± 0.53	186	76.96 ± 3.98	23.04 ± 3.98	2.69 ± 0.54
≥8 - <10	197	94.91^c ± 1.37	187	71.00 ± 4.19	29.45 ± 4.19	1.08 ± 0.54
F-value		**16.60		0.35 NS	0.35 NS	1.10 NS

NS = not significant (P> 0.05) ** P <0.01*Means with different superscripts differ significantly (P<0.05)

Table 3: The effect of storage period on fertility, hatchability, embryo mortality and chick malformation in newly hatched chicks

Storage period (days)	No. of eggs set	Mean Fertility (%) ($\bar{X}$ ± SE)	No. of fertile eggs set	*Mean Hatchability (%) ($\bar{X}$ ± SE)	*Mean Embryo mortality (%) ($\bar{X}$ ± SE)	*Mean Chick malformations (%) ($\bar{X}$ ± SE)
≥4 - ≤6	198	96.46^a ±0.51	193	70.83^a ± 2.12	28.51^a ± 1.48	0.52 ± 0.52
≥7 - ≤9	200	92.50^b ± 0.04	185	55.66^b ± 2.12	44.34^b ± 2.12	1.09 ± 1.09
≥10 - ≤14	187	78.62^c ± 1.30	147	55.75^b ± 2.05	45.96^b ± 1.60	2.72 ± 0.68
F-value		89.28**		19.15**	26.69**	0.79 NS

NS = not significant (P> 0.05) ** P <0.01*Means with different superscripts differ significantly (P<0.05)