

The impact of different pairing scheme on female reproductive investment: Research on Japanese Quail (*Coturnix japonica*)

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Abstract. Determining the sex of the embryo during incubation is crucial for the poultry industry. The primary problem to comprehend in this study is the manipulation of the sex ratio and also the egg production. The study will concentrate on how two distinct methods of pairing parents—one male with one female and another male with three females—affect egg production, the average egg mass, and the sex offspring ratio. As the animal model, we employ the layer strain of the Japanese Quail (*Coturnix japonica*) since they produce continuous eggs each day and easy to handle. The Japanese quail pairs were housed for seven days prior to the start of egg collection. These eggs were nurtured for up to three weeks after hatching until the sexing could be determined. When compared to a pair of three females, the egg production of a single pair was much higher when alternative pattern schemes were used. However, the mass of their typical eggs is remarkably similar. The male to female chick offspring sex ratio regularly showed to be 1:1 or equal. This finding could serve as the foundation for further research into the impact of partner pairing on another avian layer strain.

1 Introduction

Female chicks have a significant commercial value in the breeding sector for prolific laying poultry [1-3]. Males, meanwhile, will be eliminated shortly after hatching because they are worthless as commodities [2]. This condition not only wastes resources, but also has a detrimental effect on areas of animal care. Determining the sex of fertilized eggs as soon as possible is essential to increasing the laying poultry industry's production efficiency. [4, 5]. Investigating the mechanisms of sex control in bird breeds is another essential problem. This is significant because it can aid attempts to modify the surroundings of the female parent to create a particular sex.

Separate sexes are linked to morphological variations and the evolutionary difficulties they will encounter during the course of the species' life history [5, 6]. This has the effect of causing distinct selection pressures for the two sexes, which may change each sex's relative

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reproductive value. As a result, there are differences in the priorities of the individuals who will be selected to vary the allocation of resources [7]–[10]. Fisher made the most significant contributions to the theory of sex allocation (1930), Hamilton (1967) and Chamov (1982) [4, 5, 9, 11]. The best sex allocation by parents is anticipated to be influenced by three main process categories: differential costs, conditional sex allocation, and structured population. [11-13]

In the avian species, the female individual genetic is heterogametic (XY) and there is evidence that the female parent is able to manipulate the primary sex ratio (sex ratio at fertilization) before fertilization [6, 14]. Nonetheless, proper post-oviposition and post-hatching care is demonstrated by parental care, with both sexes of parents (male and female) participating in the upbringing of their children in many species. As a result, both parents have the ability to control the secondary sex ratio—the ratios of sexes after eggs are placed. For instance, differentiating embryonic mortality between the sexes is one way that incubation temperature influences the secondary sex ratio in birds. [10, 15, 16].

The temperature-dependent embryonic mortality of birds was first documented in the Megapode species [4, 17], the Australian turkey (*Alectura lathami*). Megapods are a unique group of birds because they use the ambient heat of decomposing plant material to incubate their eggs, as opposed to most other birds that use contact incubation, which involves the transmission of heat from the mother to the eggs by physical contact. Studies on commercial poultry have revealed secondary changes in sex ratios under varying incubation conditions, although not in an ecological context. [10, 18], and growth of male chicks that sooner after hatching under conditions where more males tend to be produced [19-21].

Based on this background, this study was intended to examine the female parent's response to changes in environmental conditions that can stimulate stress. Stress conditions will increase the level of the hormone corticosterone (CORT), which is one of the determinants of the ovulation of genetic X oocytes. This study is targeted as a first step in the study of sex modification of embryonic eggs that is economical and non-invasive. The hypothesis in this study is that the female parent will produce more eggs with different offspring sexes depending on the pairing scheme.

2 Method

2.1 Experiment Design

In Maesan Bondowoso, breeders of quail for egg and fresh meat production provided the brood stock of Japanese quail eggs for the research purpose. The eggs were incubated, and the chicks were reared for two weeks until the individual sex could be identified. The young quail, whose sex has been identified, were separated into cages containing only one sex (only male or only female). This is intended to maintain a virgin condition so that during treatment, the female parent does not recognize the sex differences of the opposite sex.

After two months of age and reaching sexual maturity, which is marked by the production of the first eggs, the male and female parents are paired. The female and male parents then recorded their biometric data, which included body weight, wing length, tarsus length, head length, and beak length. For the installation process, with a combination of the number of males and females, each individual is given a marker to facilitate randomization. After each individual has an identity along with a predetermined combination, they are kept in separate cages. The cages arrangement has been illustrated in the following figure.

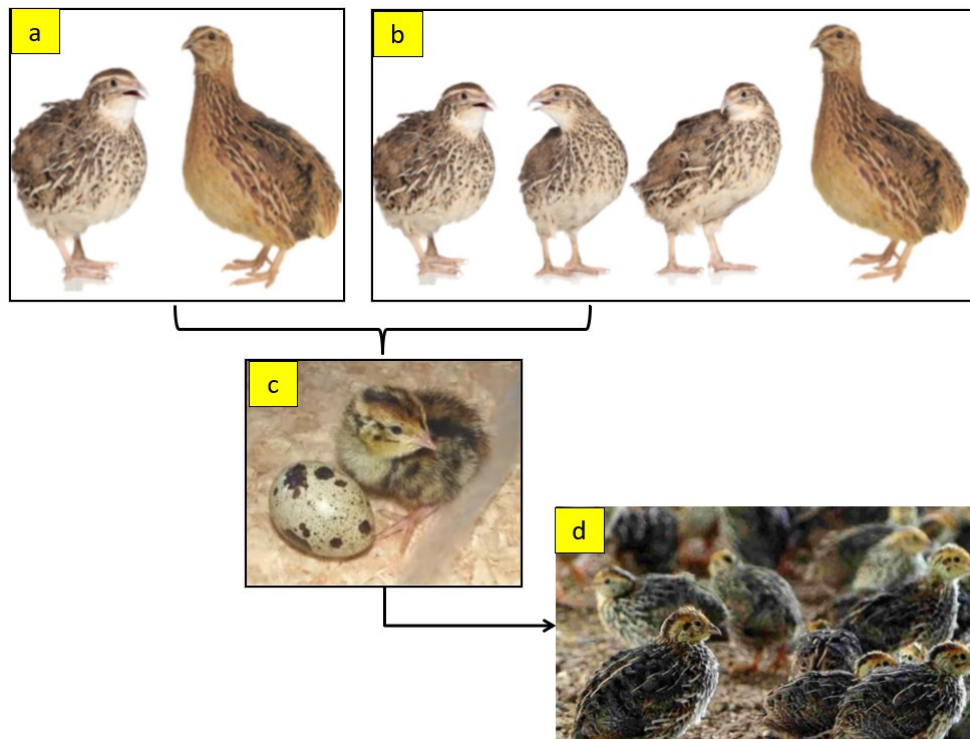


Fig 1. The arrangement of the treatment was based on the ratio of male and female parents in each cage (a and b), eggs were identified and incubated (c), and chicks were raised until the different sexes were identified (d).

After seven days of laying and rearing the brood, each egg produced is then collected and marked to distinguish its origin. Egg collection was carried out for seven days for all ratios of parent pairs. After being biometrically registered and branded, eggs are put in an incubator set at 37 degrees Celsius. For free interchange of fresh air, the eggs are positioned in alternating grooves. To maintain a balance between the yolk and albumin, the eggs are positioned horizontally and rotated every 12 hours. On the seventh day, the eggs began to be detected for their heartbeat with the "Digital Egg Monitor" monitor.

The time between retrieval in the incubator and the measurement of the embryo's heart rate carried out at room temperature should not be more than two minutes to avoid a drastic drop in egg temperatures, which adversely affect hatching success. Every day at the same time, heart rate counts of eggs are routinely performed. The hatched embryos were then marked and kept in separate cages based on the parent treatment group. After two weeks of age, the sex can be identified based on their feather pattern and behavior.

2.2 Statistical Analysis

Firstly, the biometric distribution data of all aspects of the Japanese quail, such as body mass, tarsus length, egg mass, and chick mass, will be analyzed for normality. The egg production of each pair (treatment) would be recorded based on the number of eggs after 7 days of the pairing. The productivity of eggs would be analyzed by dividing the number of eggs per treatment group each day. The average egg mass would be analyzed by dividing the egg mass by the group of treatments. The data on chicks mass at one day old was then divided by the egg mass to find the conversion point. The productivity is then analyzed using a mixed model

with each cage (treatment) as a random effect. The models include the number of eggs per treatment group and the interaction between pair identity and treatment as a fixed effect. The conversion point is then analyzed using mixed models with each chick's identity as a random effect. The models include conversion point, treatment group, and the interaction between conversion point and treatment as a fixed effect and egg mass as a covariate. The data on sex ratio between treatments would be analyzed quantitatively by regrouping all of the chicks data based on the treatment. Software called IBM SPSS Statistics 22 was used to process all of the data.

3 Result

3.1 Biometry of the parental

The biometric data analyzed was the average body mass per treatment group, there were two groups, namely one male and one female pair and one male and three female pairs. Descriptive data from the former body are as follows: Male (N=24, max=199.00, min=158.4, SE \pm 2,173, SD \pm 10,647), Female (N=24, max=216.71, min=171.4, SE \pm 3,506, SD \pm 17,177). The productivity data were analysed based on two categories i.e productivity per day and average eggs.

3.2 Eggs data

The descriptive data of both are as follow: productivity per day in three group pair (N = 13, mean = 0.689, SE \pm 0.0413, SD \pm 0.148) and single pair (N = 11, mean = 0.870, SE \pm 0.034, SD \pm 0.114), average eggs mass production of each group treatment in three group pair (N = 13, mean = 11.651, SE \pm 0.088, SD \pm 0.319) and single pair (N = 11, mean = 11.661, SE \pm 0.213, SD \pm 0.709). The analyze data of average eggs showed that both treatment has no significant different $F = 0.005$, $p = 0.945$; the male body mass has no contribution to the average eggs mass $F = 0.22$, $p = 0.883$, however the female body mass has significant effect to the average of eggs mass $F = 14,824$, $p = 0,001$. The analyze data of productivity eggs per days showed that both treatment has significant different $F = 8.677$, $p = 0.08$; the male body mass has no contribution to the average eggs mass $F = 0.183$, $p = 0.673$, it also imply in the female body mass which has no significant effect to the productivity of eggs per day $F = 0.065$, $p = 0,801$.

3.3 Chicks data

The number of survive chicks when the sex could be determined in three pairs group are 95 chicks (49 females and 46 males) where in the single pairs group are 43 chicks (21 females and 22 males). In total, there are 138 chicks with the ratio of male and female is 70 : 68 or near 1 : 1.

4 Discussion

We study the difference in the avian sex ratio in two different parental pairing setups: first in single males and females, and second in single males and three females. The result showed that females produce more eggs when housed in single pairs than in three females. The productivity of single pairs is significantly higher than that of three females, as shown in figure 2.

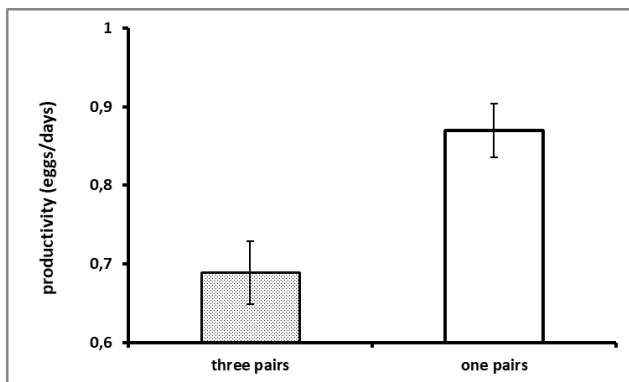


Fig 2. The variations in Japanese quail egg productivity between one pair and three pairs. Three pairs generate less eggs a day than one pair, whose females produce almost one egg a day.

This result showed that females could produce more eggs in single pairs than three pairs; it seems that she was already able to adjust to the stress stimulated by the aggressive behavior of the male. The females in single pairs continue to produce eggs daily, and the three pairs could not perform stable egg production. [22, 23]. This could be stimulated by the competition between females to utilize resources such as food or space [22, 24]. Another factor could be coming from the stress due to the aggressiveness of the male in crowded condition. Based on the behavioral observation, we found that males in three pairs show more aggression than in single pairs [22, 25]. This could be stimulated by the presence of many females, where the male pursues to produce more ejaculate to mate with all of the females [26, 27]. This situation could increase the level of testosterone in the circulation of male quail while at the same time increasing the level of corticosterone in the circulation of females [16, 28]. However, we lack information regarding hormone levels during housing time.

As Figure 3 illustrates, the average egg mass of the two matching methods was comparable. Here, we found that females produce relatively similar egg masses, no matter their pairing scheme. There is an interesting fact that female body mass contributes to egg mass, as shown in the statistic.

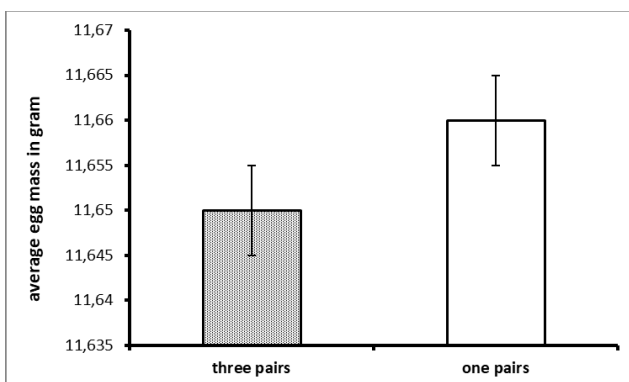


Fig 3. The average egg mass in two different pairings The figure showed that both schemes have an impact on egg mass. However, the average egg mass in three pairs is relatively lighter than in one pair (but not statistical).

This result shows that the egg mass would be dependent on the female body mass. Since the random choice of the sample individual would put all of the females of different body masses into the experiment. At the same time, there was no effect of male body mass on the

egg mass. Only female's control the egg mass; the only significant influence on egg quality is the number of females per male in each cage. Females generate generally identical egg masses because the sex of the embryo determines the hormonal content of fertile vs sterile eggs, and the generation of embryonic hormones alters the concentration of maternal hormone. [29].

Endogenous hormones started to manufacture with the development of the embryo, primarily in the gonads. Gonadal development, in which the female embryo already generates an oocyte, may have driven the greater T content in the embryo. [21]. Groothuis has reviewed the role that androgen plays in the development of the embryo. [30]. Alpha-dihydrotestosterone (DHT), androstenedione (A4), which have many biological activities and can have significant effects during development, are members of this category. Mothers may first employ these androgens as a tool to manipulate the embryo's development in order to modify its phenotype to fit current or upcoming environmental circumstances. [31, 32]. Increased yolk androgen levels can have a range of consequences on the physiology, immune system, behavioral phenotype, developmental time, and growth of the chicks; some of these effects are beneficial, while others, such as immune system and metabolic rate, are unfavorable [33–38].

In chick data, we found that both schemes of pairing produce a relatively similar proportion of male and female chicks. All of the surviving chicks share the same individual (70:68, or near 1:1), which demonstrates that mother condition did not influence the production of female or male chicks. We assume both sexes follow a similar scenario and survive with the same probability that the mother delivers the same proportion of ovum with the W or Y genes, which in turn share an equal ratio. This result stimulates the possibility that the mother is still able to overcome the stress in the presence of males and females by producing the same proportion of male and female offspring [11, 39, 40]. Or it could be possible that the level of stress and corticosterone circulation remain below the threshold of the mother's ability to adjust. It would be valuable if we could find the threshold and stimulate it to study how mothers respond in proportion to their offspring.

5 Conclusion

In conclusion, our results suggest that the avian sex ratio remains constant when the mother is stimulated by two different pairs. The stress of being paired with single males did not change the sex ratio of the offspring but rather stimulated the mother to produce more eggs than pairing with three females. These findings open opportunities for additional research on several bird species, including domestic chicken, particularly in the context of the poultry industry.

A.L. Y.N and B.S. designed the experiment. A.L. performed the experiments. A.L. and Y.N analyzed the data. A.L. wrote the first draft of the manuscripts. A.L. is writing the final version of the document. All authors read and approved the final manuscript. The authors declare to have no potential conflicts of interest (financial or nonfinancial) that are directly or indirectly related to the research. We thank Efi Fadjriah for the supporting of all aspect in the experiment and administrative procedure.

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