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Project Title: Parental sex effect of parthenogenesis on egg weight in mated Chinese Painted quail

Abstract: Parthenogenesis (P), embryonic development in unfertilized eggs, occurs in poultry. In virgin quail, correlation analysis revealed that parthenogen size increases as initial egg set weight increases. Additionally, in mated hens, P negatively impacts hatchability. Perhaps elevated egg set weight is a causative factor for decreased hatchability in hens exhibiting P. Egg set weight in birds exhibiting P may influence embryonic mortality and other hatching failures in mated hens. Also, it is unknown if P in the hen, her mate or both impact egg set weight in mated Chinese Painted quail. Therefore, the objective of this study was to determine if selection for P in the hen, her mate or both influences egg set weight for eggs that hatch as well as eggs that ultimately yield various hatching failures. Females and males used in the current study consisted of 2 genetic lines of birds, one selected for P and one not selected for P (controls, C). A 2 hen (C and P) x 2 male (C and P) factorial arrangement of breeding pair treatments was utilized to determine the impact of P on egg set weight for eggs that hatched as well as those that failed to hatch. Hatching failures were classified as infertile, P, early embryonic mortality, or late embryonic mortality. The 4 breeding pair treatments were as follows: C hens with C males (CC), C hens with P males (CP), P hens with C males (PC), and P hens with P males (PP). Daily, eggs were collected, labeled and weighed prior to incubation at 37.5°C for 18 d. All eggs that didn't hatch were broken to determine hatching failures. Egg set weight for eggs that hatched was greater ($P < .05$) when the hen or male exhibited P as compared to eggs from C birds. However for infertile eggs, a hen by male interaction revealed that set weight was higher in PP when compared to PC, CP and CC eggs. For eggs that yielded early and late embryonic mortality, set weight for both hatching failures was greater in P as compared to C hens. In conclusion, it appears that egg set weight is heaviest when eggs are from P hens regardless of hatching failure. More interestingly, not only P hens, but also P males appear to influence set weight of eggs that hatch, perhaps by altering embryonic development.

PARENTAL SEX EFFECT OF SELECTION FOR
PARTHENOGENESIS ON EGG WEIGHT AT
SET IN MATED CHINESE PAINTED QUAIL

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Parthenogenesis (P), embryonic development in unfertilized eggs, occurs in poultry. In virgin quail, correlation analysis revealed that parthenogen size increases as initial egg set weight increases. Additionally, in mated hens, P negatively impacts hatchability. Perhaps elevated egg set weight is a causative factor for decreased hatchability in hens exhibiting P. Egg set weight in birds exhibiting P may influence embryonic mortality and other hatching failures in mated hens. Also, it is unknown if P in the hen, her mate or both impact egg set weight in mated Chinese Painted Quail. Therefore, the objective of this study was to determine if selection for P in the hen, her mate or both influences egg set weight for eggs that hatch as well as eggs that ultimately yield various hatching failures. Females and males used in the current study consisted of 2 genetic lines of birds, one selected for P and one not selected for P (controls,C). A 2 hen (C and P) x 2 male (C and P) factorial arrangement of breeding pair treatments was utilized to determine the impact of P on egg set weight for eggs that hatched as well as those that failed to hatch at $P < 0.05$. Hatching failures were classified as infertile, P, early embryonic mortality, or late embryonic mortality. The 4 breeding pair treatments were as follows: C hens with C males (CC), C hens with P males (CP), P hens with C males (PC), and P hens with P males (PP). Daily, eggs were collected, labeled and weighed prior to incubation at 37.5 °C for 18 d. All eggs that didn't hatch were broken to determine hatching failures. Egg set weight for eggs that hatched was greater ($P < 0.05$) when the hen or male exhibited P as compared to eggs from C birds. However for infertile eggs, as well as for eggs that yielded early and late embryonic mortality, set weight for these hatching failures was greater in P as compared to C hens. In conclusion, it appears that egg set weight is heaviest when eggs are from P hens regardless of hatching failure. More interestingly, not only P hens, but also P males appear to influence set weight of eggs that hatch, perhaps by altering embryonic development.

General Introduction of Parthenogenesis:

Parthenogenesis, embryonic development of an unfertilized egg, occurs in turkeys, chickens [1], zebra finches [3] and Chinese Painted quail [4]. It was discovered in Beltsville Small White Turkeys by M.W. Olsen in 1953 when approximately 14 percent of the unfertilized eggs he collected developed after 48 hours of incubation. Later in 1998, Cassar et al [2] used the same line of turkeys as Olsen to find that around 30 percent of the unfertilized eggs in his study developed embryos and .3 percent hatched. It is estimated that today around 4 percent of commercial turkey eggs contain parthenogens [9]. Parthenogenesis has also been known to occur in approximately 4 percent of the infertile eggs laid by the most popular bred of chickens, the Cornish, used for the production of meat [1].

Parthenogens are almost perfectly identical to their mothers because they have almost the same genetic makeup, and all that hatch are male. Parthenogenesis in Chinese Painted Quail was discovered in the mid-2000s at the Mississippi State University Poultry Science Department by Dr. Chris McDaniel and Dr. Holly Parker as they were breeding them for an Avian Reproduction Laboratory [10].

Research Objective:

To determine if selection for parthenogenesis in the hen, her mate or both influences egg set weight for eggs that hatch as well as eggs that ultimately yield various hatching failures.

Research Introduction:

Parthenogen embryonic size increases as initial egg set weight increases in virgin quail hens [5]. Parthenogenesis also negatively affects hatchability in mated hens [6], and it is known that

infertile eggs from mated hens contain parthenogens [7]. Because there is a positive relationship between initial egg set weight and parthenogen size in virgin quail, possibly egg set weight is altered in mated hens exhibiting parthenogenesis which reduces hatchability. This reduction in hatchability may be due not only to the impact of egg set weight on parthenogenetic development but also the impact of egg weight on fertility as well as early and late embryonic mortality. If egg set weight does influence hatchability, which parental sex, the dam or sire, is most responsible for the alteration in egg set weight seen when birds exhibit parthenogenesis.

Methods and Materials:

Two lines of Chinese Painted quail (88 total) were utilized: one not selected for parthenogenesis (Control, C) and the other selected for over 6 generations for the parthenogenetic (P) trait [6]. A 2 hen (C and P) x 2 male (C and P) factorial arrangement of breeding pair treatments was utilized (Table 1 as shown on the following page). Daily for 10 months, eggs (2,748 total) were collected, labeled, and weighed according to their treatment. Eggs were incubated at 37.5 °C for 18 days. After 18 days of incubation, all eggs that did not hatch were broken open to determine when the hatching failure occurred. Unhatched eggs were classified as: parthenogen, infertile, early dead, middle dead, late dead, cracked, or contaminated. Data were analyzed as a completely randomized design with individual breeding pairs (11 per treatment combination) serving as experimental units.

Table 1. Breeding Pair Treatment Combinations			
		Dam Line	
		Control	Parthenogenetic
Sire Line	Control	CC	CP
	Parthenogenetic	PC	PP

Results:

As compared to the controls, the dams that were selected for parthenogenesis exhibited up to 13% higher initial set weights for eggs yielding: parthenogens (Figure 1 shown on bottom of this page), infertility (Figure 2 shown on bottom of this page), early embryonic mortality (Figure 3 shown on following page), and late embryonic mortality (Figure 4 shown on following page). However, the initial egg set weight for the eggs that hatched was higher when either the dams (Figure 5 shown on following page) or sires were selected for the parthenogenetic trait (Figure 6 shown on following page).

There were no significant interactions detected between dam and sire ($p > .10$).

Statistical Graphs:

Figure 1. Main effect of Dam on set weight of eggs that contained Parthenogens

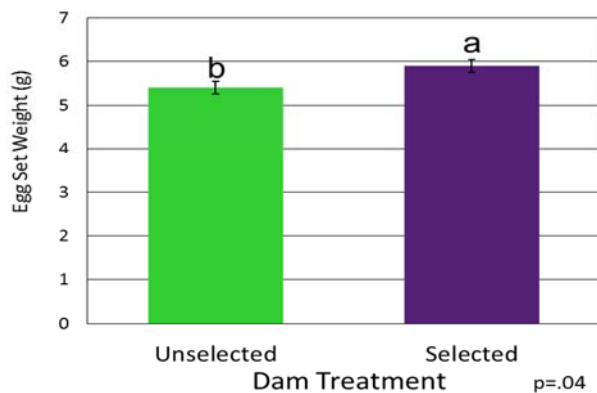


Figure 2. Main effect of Dam on set weight of eggs that were Infertile

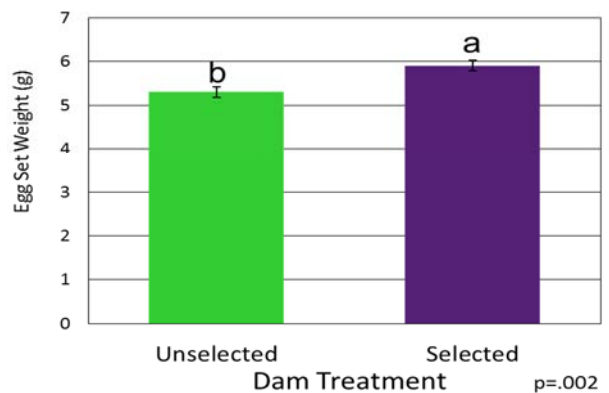


Figure 3. Main effect of Dam on set weight of eggs with Early Embryonic Mortality

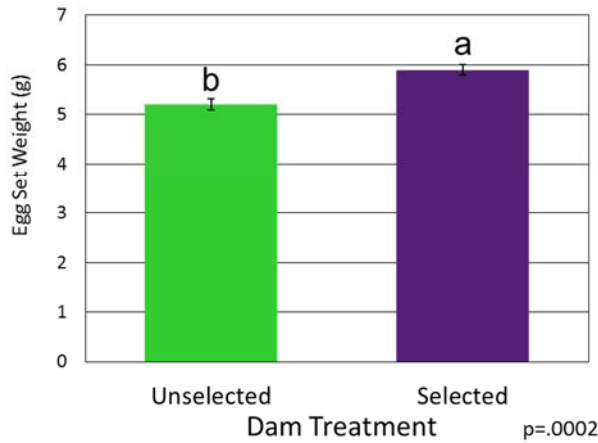


Figure 4. Main effect of Dam on set weight of eggs with Late Embryonic Mortality

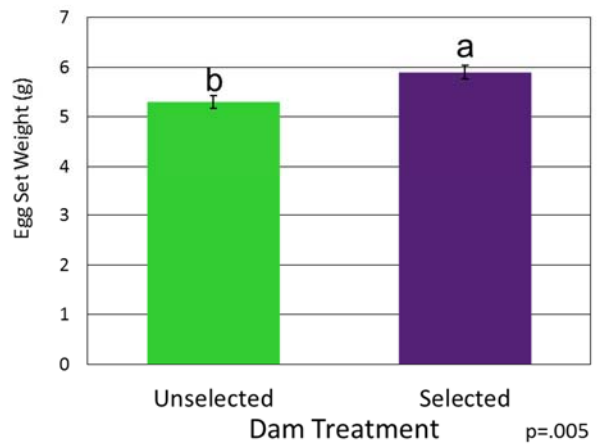


Figure 5. Main effect of Dam on set weight of eggs that Hatched

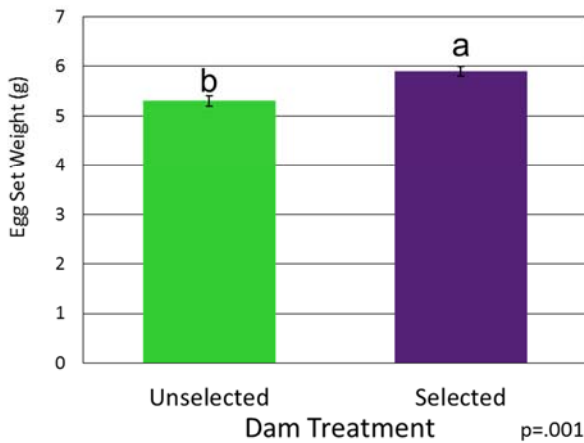
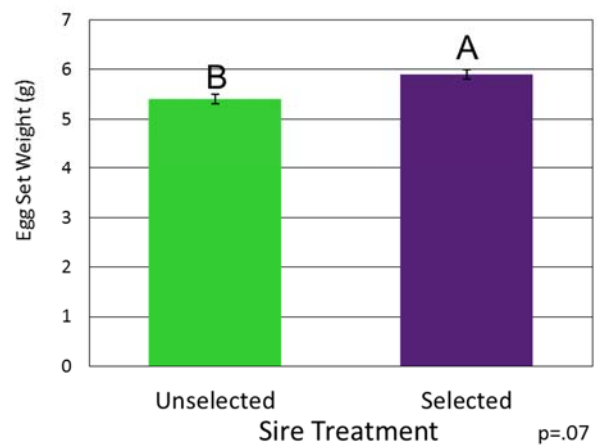


Figure 6. Main effect of Sire on set weight of eggs that Hatched



Discussion:

Higher initial egg set weight, due to selection of dams for parthenogenesis and not sires, may be why hatchability is negatively impacted in birds exhibiting parthenogenesis. Apparently, this higher initial set weight is most influential in eggs that don't hatch due to parthenogen formation, infertility, early deads, and late deads. Because eggs from selected dams have a higher set weight as well as lower egg production and shorter egg clutches[4], it is possible that the ova from selected dams are larger due to extended development on the ovary. This delayed ovarian

follicular development may result in, not only, larger ova and hence larger eggs at lay [8] but also abnormal embryonic development. The increase in set weight when sires were selected for the parthenogenetic trait is perplexing. Perhaps embryos resulting from fertilization by selected males may alter albumen secretion or the length of egg formation in the hen and therefore yield larger eggs.

Student Experience:

As being a part of the research team that conducted a study on Chinese Painted Quail to determine which sex when selected for parthenogenesis had the greatest effect on egg set weight, I learned many new things. I was able to learn how to keep accurate records documenting what had been done step by step in this research and the importance behind it. I also learned that research in any format is time consuming and that in order for the statistical analysis to work properly all data must be entered into the computer properly. I learned to candle eggs after 10 days of incubation using a hand candler to determine whether or not the eggs showed any formation of an embryo. I also used this method to separate the eggs that did not exhibit any embryonic formation from those that did in order for the eggs to be broken open to determine failure and to continue the incubation of those that did have embryos. I also learned how to operate the incubators and the importance of keeping them at 100 F and filled with water if you want to have a successful hatch. Sperm egg penetration was the most time consuming thing I learned how to do, and I found it to be different than what I had previously learned how to do in an Avian Reproduction lab. Each part of the egg is broken down and weighed separately. The outer covering of the yolk is affixed to a slide and stained with Schiff's reagent and formalin so that sperm penetration holes will become visible under the microscope. A grid on the microscope

eyepiece is used to determine the number of holes made by the sperm in a certain area. All of the weights and number of penetrations are recorded for proper documentation.

Learning Outcomes:

I was able to expand my knowledge of parthenogenesis that I had first learned about as it was introduced to me in the Avian Reproduction and Genetics classes that I had previously taken at Mississippi State University. I was also able to become aware of how much parthenogenesis as a whole could unknowingly affect the poultry industry in which I am pursuing a career.

Parthenogenesis is widely known in the turkey industry, but very few people know about it in the chicken side of the poultry industry. It occurred to me while working on this research that what hatcheries may consider as very early dead embryos, that did not hatch after 21 days of incubation, could in fact be parthenogens from unfertilized eggs.

I also used my knowledge of how parthenogenesis occurs in other species to make an educated guess that parthenogenesis could be a natural occurrence that keeps a species from going revolutionarily extinct. The male parthenogens that survive long enough to reproduce can mate with the available females and keep the genetics and species going.

Deliverables Achieved:

After receiving a scholarship to conduct undergraduate research through the College of Agriculture and Life Sciences (CAL S) and Mississippi Agriculture and Forestry Experiment Station (MAFES) in the early part of September, I was able to conduct research under one of the

Mississippi State University Poultry Science Department professors on the topic of Parthenogenesis in Chinese Painted Quail. I was also able to present a scientific poster on the work I had done while at the International Poultry Scientific Forum held in Atlanta, Georgia this past January. In February, I presented a PowerPoint presentation to my seminar class informing them of what I had learned in such a short period of time after starting this research in September. In April, I will be presenting my work at the Honors Research Symposium on the Mississippi State University campus.

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- [5] Wells, J. B., H. M. Parker, A. S. Kiess, and C. D. McDaniel, 2012. The relationship of incubational egg weight loss with parthenogenesis in Chinese Painted quail (*Coturnix chinensis*). Poultry Sci, 91: 189-196.
- [6] Parker, H. M., A. S. Kiess, P. S. Rosa, D. Rowe and C. D. McDaniel. 2014. Selection for the parthenogenetic trait in Chinese Painted Quail (*Coturnix chinensis*) and its effect on hatchability parameters by generation. Poultry Sci 93:664-672.
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[8] Wolanski, N. J., R. A. Renema, F. E. Robinson, V. L. Carney, and B. I. Fancher, 2007. Relationships among egg characteristics, chick measurements, and early growth traits in ten broiler breeder strains. *Poultry Sci* 86:1784–1792.

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PARENTAL SEX EFFECT OF SELECTION FOR PARTHENOGENESIS ON EGG WEIGHT AT SET IN MATED CHINESE PAINTED QUAIL

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Abstract (P237)

Parthenogenesis (P), embryonic development in unfertilized eggs occurs in poultry. In virgin quail, correlation analysis revealed that parthenogen size increases as initial egg set weight increases. Additionally, in mated hens, P negatively impacts hatchability. Perhaps elevated egg set weight is a causative factor for decreased hatchability in hens exhibiting P. Egg set weight in birds exhibiting P may influence embryonic mortality and other hatching failures in mated hens. Also, it is unknown if P in the hen, her mate or both impact egg set weight in mated Chinese Painted Quail. Therefore, the objective of this study was to determine if selection for P in the hen, her mate or both influences egg set weight for eggs that hatch as well as eggs that ultimately yield various hatching failures. Females and males used in the current study consisted of 2 genetic lines of birds, one selected for P and one not selected for P (controls, C). A 2 hen (C and P) x 2 male (C and P) factorial arrangement of breeding pair treatments was utilized to determine the impact of P on egg set weight for eggs that hatched as well as those that failed to hatch at $P < 0.05$. Hatching failures were classified as infertile, P, early embryonic mortality, or late embryonic mortality. The 4 breeding pair treatments were as follows: C hens with C males (CC), C hens with P males (CP), P hens with C males (PC), and P hens with P males (PP). Daily, eggs were collected, labeled and weighed prior to incubation at 37.5°C for 18 d. All eggs that didn't hatch were broken to determine hatching failures. Egg set weight for eggs that hatched was greater ($P < 0.05$) when the hen or male exhibited P as compared to eggs from C birds. However for infertile eggs, a hen by male interaction revealed that set weight was higher in PP when compared to PC, CP, and CC eggs. For eggs that yielded early and late embryonic mortality, set weight for both hatching failures was greater in P as compared to C hens. In conclusion, it appears that egg set weight is heaviest when eggs are from P hens regardless of hatching failure. More interestingly, not only P hens, but also P males appear to influence set weight of eggs that hatch, perhaps by altering embryonic development.

Key words: Parthenogenesis, hatchability, egg weight, fertility, embryonic mortality

Introduction

Parthenogenesis, embryonic development of an unfertilized egg, occurs in turkeys, chickens [1], zebra finches [2] and Chinese Painted quail [3]. Parthenogen embryonic size increases as initial egg set weight increases in virgin quail hens [4]. Parthenogenesis also negatively affects hatchability in mated hens [5], and it is known that infertile eggs from mated hens contain parthenogens [6]. Because there is a positive relationship between initial egg set weight and parthenogen size in virgin quail, possibly egg set weight is altered in mated hens exhibiting parthenogenesis which reduces hatchability. This reduction in hatchability may be due not only to the impact of egg set weight on parthenogenetic development but also the impact of egg weight on fertility as well as early and late embryonic mortality. If egg set weight does influence hatchability, which parental sex, the dam or sire, is most responsible for the alteration in egg set weight seen when birds exhibit parthenogenesis.

Objective:

To determine if selection for parthenogenesis in the hen, her mate or both influences egg set weight for eggs that hatch as well as eggs that ultimately yield various hatching failures.



Hatching Failures



Parthenogen



Infertile



Early Dead



Late Dead

Hatched Chick



Table 1. Breeding Pair Treatment Combinations

		Dam Line	
		Control	Parthenogenetic
Sire Line	Control	CC	CP
	Parthenogenetic	PC	PP

Figure 1. Main effect of Dam on set weight of eggs that contained Parthenogens

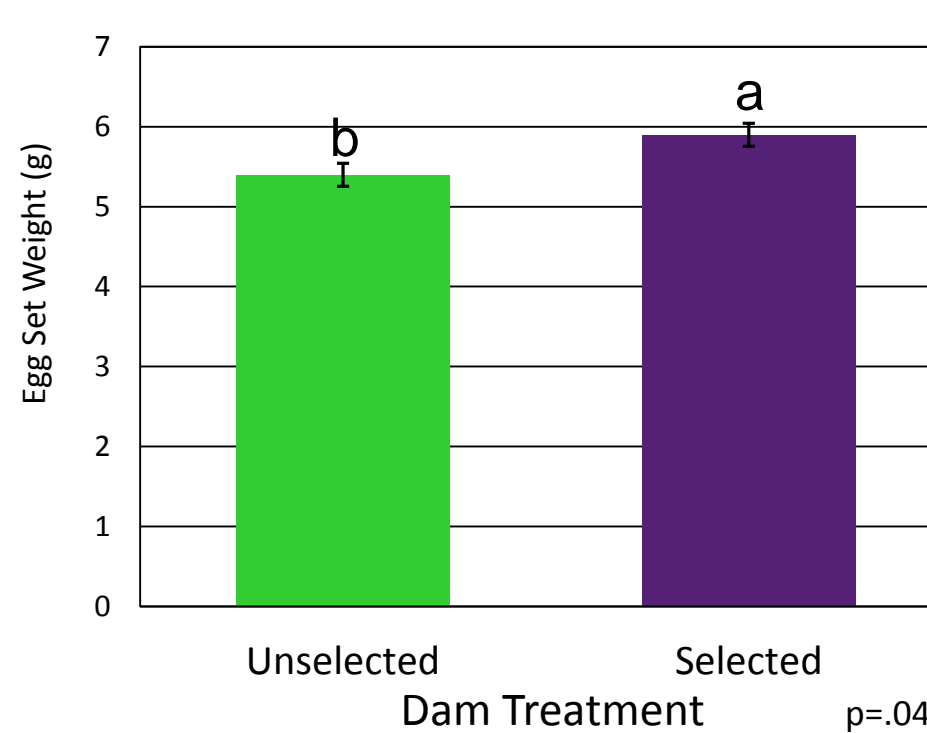


Figure 2. Main effect of Dam on set weight of eggs that were Infertile

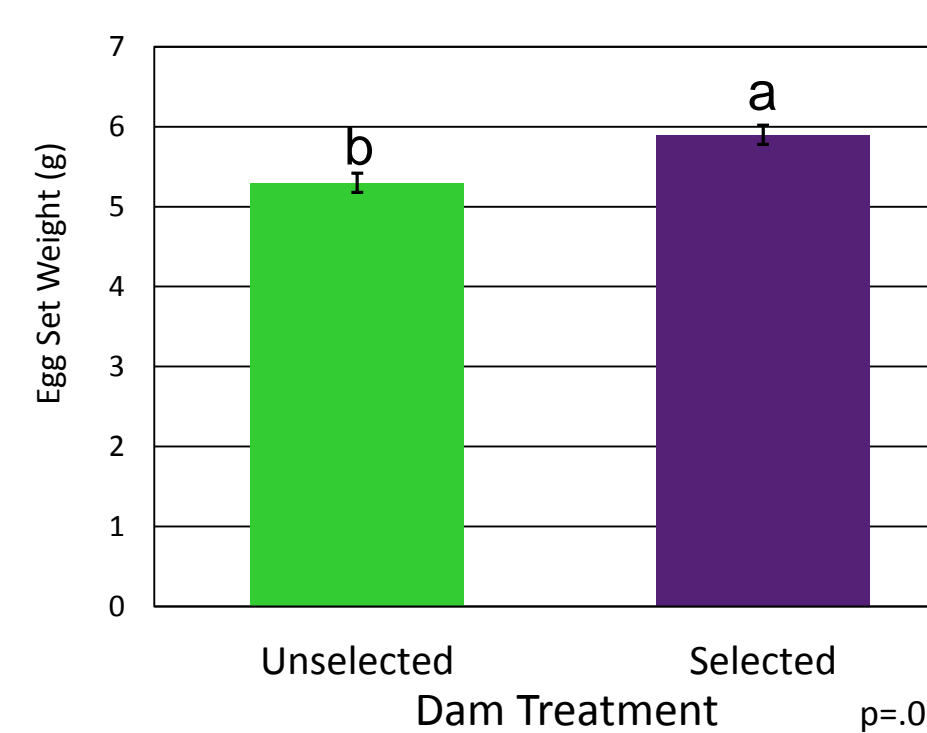


Figure 3. Main effect of Dam on set weight of eggs with Early Embryonic Mortality

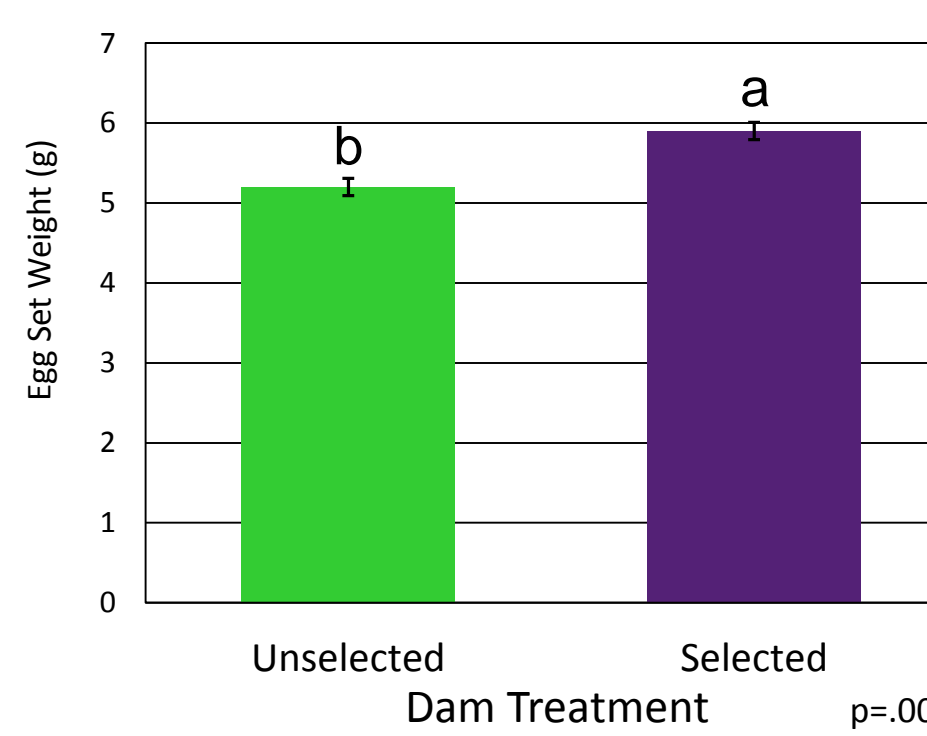


Figure 4. Main effect of Dam on set weight of eggs with Late Embryonic Mortality

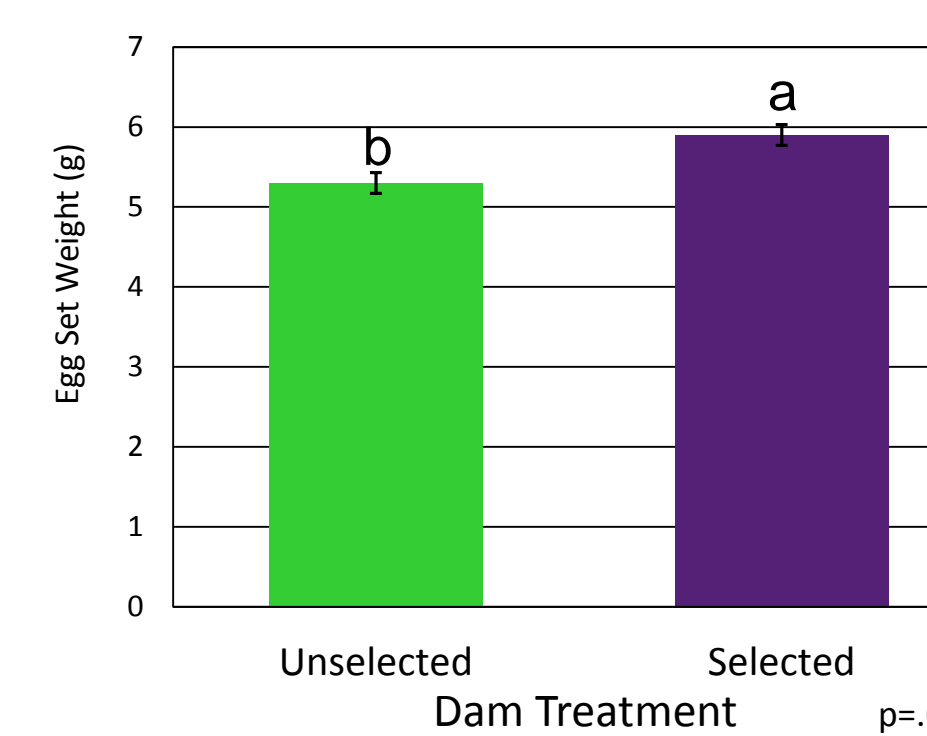


Figure 5. Main effect of Dam on set weight of eggs that Hatched

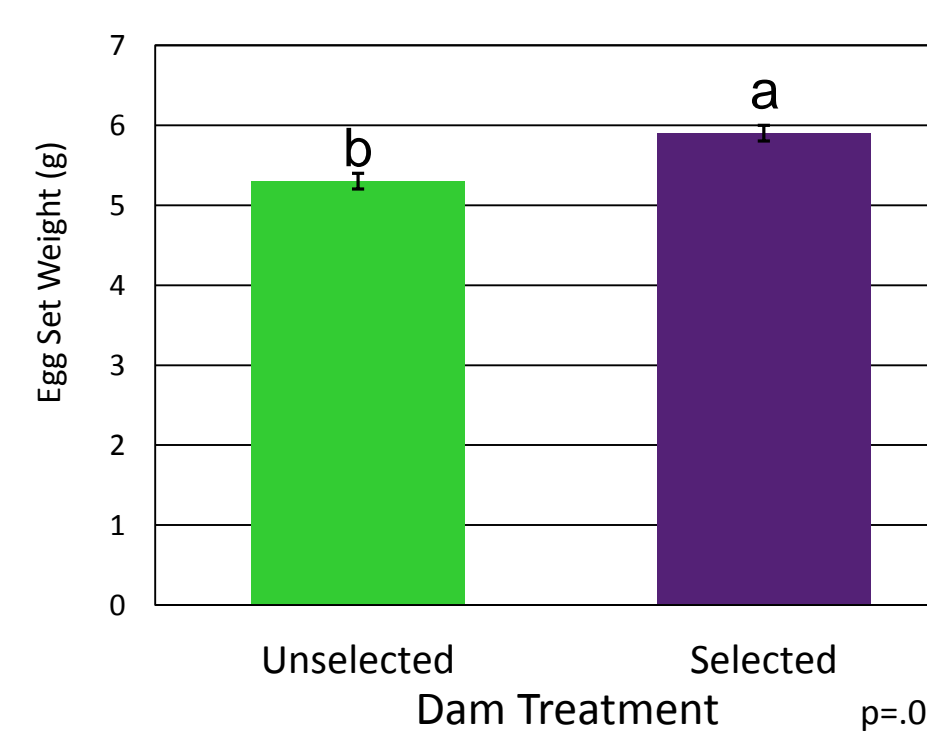
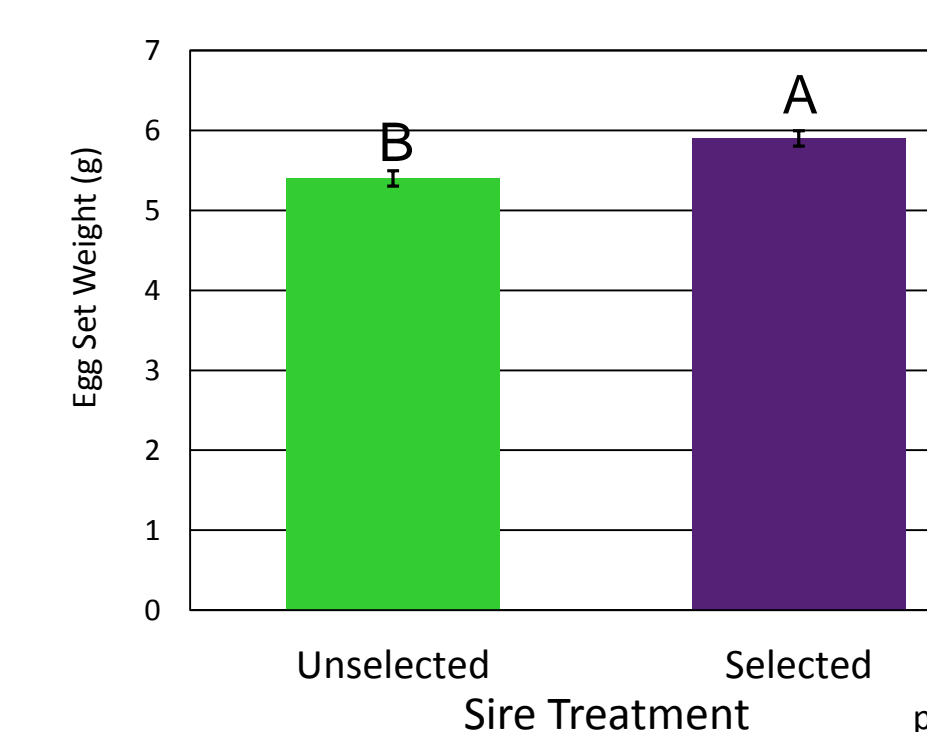


Figure 6. Main effect of Sire on set weight of eggs that Hatched



Materials and Methods

Two lines of Chinese Painted quail (88) were utilized: one not selected for parthenogenesis (Control, C) and the other selected for over 6 generations for the parthenogenetic (P) trait [5]. A 2 hen (C and P) x 2 male (C and P) factorial arrangement of breeding pair treatments was utilized (Table 1). Daily for 10 months, eggs (2,748) were collected, labeled, and weighed. Eggs were incubated at 37.5°C for 18 d. After 18 d of incubation, all eggs that did not hatch were broken open to determine when the hatching failure occurred. Unhatched eggs were classified as parthenogen, infertile, early dead, middle dead, late dead, cracked, or contaminated. Data were analyzed as a completely randomized design with individual breeding pairs (11 per treatment combination) serving as experimental units.

Results

As compared to controls, dams selected for parthenogenesis exhibited up to 13% higher initial set weights for eggs yielding:

- parthenogens (Figure 1)
- infertility (Figure 2)
- early embryonic mortality (Figure 3)
- late embryonic mortality (Figure 4)

However, initial egg set weight for eggs that hatched was higher when either dams (Figure 5) or sires were selected for the parthenogenetic trait (Figure 6).

No significant interaction was detected between dam and sire ($p > .10$).

Discussion

Higher initial egg set weight, due to selection of dams for parthenogenesis and not sires, may be why hatchability is negatively impacted in birds exhibiting parthenogenesis. Apparently, this higher initial set weight is most influential in eggs that don't hatch due to parthenogen formation, infertility, early deaths, and late deaths. Because eggs from selected dams have a higher set weight as well as lower egg production and shorter egg clutches [3], it is possible that the ova from selected dams are larger due to extended development on the ovary. This delayed ovarian follicular development may result in, not only, larger ova and hence larger eggs at lay [7] but also abnormal embryonic development.

The increase in set weight when sires were selected for the parthenogenetic trait is perplexing. Perhaps embryos resulting from fertilization by selected males may alter albumen secretion or the length of egg formation and therefore yield larger eggs.

References

- [1] Olsen, M. W. and S. J. Marsden. 1953. Development in unfertilized turkey eggs. J. Esp. Zool. 126:337-346.
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