5-4-2002

The Effects of Albumen Removal on Broiler Breeder Eggs Prior to Incubation on Hatchability and Chick Weight

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An Upper Division Honors Thesis
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May 4, 2002

Submitted to:
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Abstract

In this experiment, two trials were conducted testing the relationship between albumen withdrawal and hatchability, and the relationship between albumen removal and chick weight. The experiment tested the effects of the removal of zero, one, two, or three mL of albumen on the hatchability and chick weight of broiler breeders prior to incubation. Neither trial showed a difference between the control groups and the treatment groups for egg weights, chick weights, or hatchability, but a significant difference was seen in the ratio of chick weight to egg weight for eggs with albumen removed. The results of this experiment bolster previous findings on the affects of albumen removal on hatchability, but also suggest that the ratio of chick weight to egg weight does suffer when albumen is extracted from the egg.

Introduction

Much of what is understood about the avian egg, its components, the chemical composition of the parts and their contribution to embryo development can be attributed to the monumental work of Alexis and Anastasia Romanoff, a husband and wife team that published a wealth of findings in three volumes entitled The Avian Egg, The Avian Embryo and The Biochemistry of the Avian Embryo. Their work also illuminates the effect that changes in the internal and external environments of the egg have on embryo growth, hatchability, and chick development. From within the confined and protected environment of the egg, the developing embryo receives all of the nutrition, proteins, vitamins and minerals it needs in order to divide, differentiate, and become a chick. According to the Romanoff's, "the development of the avian embryo depends entirely upon the reserve nutrients of the egg—the yolk, the albumen, and, to some extent, the shell (Romanoff and Romanoff, 1967: 179)."

The three main components of the avian egg are the yolk, the albumen and the shell. Comparing total egg weight to the weights of its constituent parts, a hen's egg is about six parts albumen, three parts yolk, and one part shell, or about 56% albumen, 32% yolk, and 12% shell (Romanoff and Romanoff, 1949: 115). The egg yolk provides the developing embryo with all of the lipids, phospholipids and other fat soluble dietary substances it needs. It also contains proteins, and to a much lesser extent, carbohydrates, electrolytes, and vitamins (Romanoff and Romanoff, 1967: 181). The albumen of the egg represents twice the volume of the yolk, holds three times more water, one-half as much solid matter, and an equal amount of proteins as the egg yolk (Romanoff and Romanoff, 1949: 315, and Romanoff and Romanoff, 1967: 181).

The main constituent of albumen is water, but the solutes dissolved within it and the other solid particles of the albumen, while a smaller part of the overall composition of the albumen by weight and percentage of total volume, play an immensely important role in the development of the chick embryo. Albumen contains twice the amount of carbohydrates as the yolk (mostly in the form of bound sugar, followed by concentrations of free glucose and, lastly, glycogen) (Romanoff and Romanoff, 1967: 192,193). According to the Romanoff's, these carbohydrates, "enter into the structure of new tissues in the developing embryo and assist in the functional activities of the organism (Romanoff and Romanoff, 1949: 322)." The albumen also contains several vitamins and minerals including: ascorbic acid, vitamin A, choline, sulphur, chloride, sodium, potassium, phosphorus, magnesium, and calcium, as well as trace amounts of copper and iron (Romanoff and Romanoff, 1967: 192, 193). The major albumen proteins, making up 86% of the solids in albumen, in order of highest concentration to lowest concentration are: ovalbumin, ovomucoid, ovoconalbumin, ovoglobulin, and ovomucin (Romanoff and Romanoff, 1967: 191).

According to The Avian Egg, the major and most important component of egg albumen is water. The water in the egg holds the various salts, proteins, carbohydrates, and emulsified fats in solution thereby making possible all the chemical activities of the egg (Romanoff and Romanoff, 1949: 322). Aside from water's ability to act as a solvent, its other special properties also provide special roles in the protection and development of the embryo. Water is able to resist changes in temperature, protecting the embryo from violent temperature changes, and water's high surface tension is important for the conservation of cell boundaries during embryo cell division and differentiation

(Romanoff and Romanoff, 1949: 323, 324). In addition to food and water, the albumen also provides physical and microbial protection to the embryo (Romanoff and Romanoff, 1967: 179).

Studies manipulating the levels of albumen in an egg can be helpful in better understanding the relationship between the internal environment of the egg and chick development. Beginning in 1959 and lasting a period of 16 years, a team of Japanese scientists studied this relationship and some of the specific biological and physiological effects that altering the internal environment of the egg has on chick livability and performance. Their work involved different trials on albumen removal and the transfer of albumen from the egg of one species to eggs of another (Arai, Mizuma, and Nishida, 1975).

Today, new implications for the relationship between albumen and embryo development are being tested by researchers. Because of the size of the poultry industry and the extensive use of poultry for medicinal and health research, improving bird performance can lead to improvements for the commercial poultry businesses, human and animal nutrition, the environment, and even human health. In attempts to make all possible improvements in bird performance, some researchers have begun to manipulate egg albumen despite its vital and well-understood importance in chick development.

Transgenics is one example of a type of research that can require egg albumen manipulation. The field of transgenic research is growing at accelerated rates and the possibilities that breakthroughs in those areas can provide are highly attractive from scientific perspectives, world health and nutrition standpoints, and from the perspective of the profit-oriented private sector. But, in order for researchers to manipulate the

genetic make up of chick embryos they must often remove a portion of the albumen to expose the nucleus of the inchoate embryo. Only with the albumen removed can the embryo then be positioned so that it can be easily altered by researchers.

This experiment examines the relationship between the extraction of egg albumen and resultant hatchability and chick weight. Because albumen is so essential to embryo development and, consequently, future bird performance and livability, there needs to be parameters established outlining the effects that different levels of albumen removal have on chick hatchability and performance. This experiment examines the connection between albumen removal and chick weight as well as the effects of albumen removal on hatchability. In the first trial, two control groups were used to determine the effect of the operation itself on hatchability. Data also were recorded on egg weight, chick weight, and the ratio of chick weight to egg weight for the four treatment groups. Together with Trial 1, Trial 2 aimed to further establish a correlation between chick weight and egg weight in eggs with albumen extracted from them and to provide more information on the relationship between albumen removal and hatchability.

Literature Review

Egg Size and Chick Weight:

The possible relationship between egg weight and chick weight has interested those in the poultry industry for years. A study by Halbersleben and Mussehl in 1921 showed a positive relationship between egg weight, chick weight, and hatching time. They also found that chick weight was nearly 64% of the unincubated weight of the egg (qtd. in Bray and Iton, 1962). In 1928, Upp published results indicating that chick weight was 68% of egg weight and that a chick's growth rate was related to chick size at hatch (qtd. in Coleman, Siegel, and Siegel, 1964). Again, in 1930, Jull and Heywang concluded that chick weight was 68% of egg weight (qtd. in Bray and Iton, 1962). Later in 1935, Blunn and Gregory and Krzanowska published findings contrary to those of previous researchers. Their results indicated that egg weight did not influence embryo weight (qtd in Zervas, 1964). But McNally and Byerly in 1936 and 1938 respectively, showed once more that egg weight did influence embryo weight (qtd in Zervas, 1964). Wiley discovered in 1950 that not only did a connection exist between egg weight and chick weight, but the relationship between the two decreased as chicks aged, remaining highly significant until 9 weeks of age (qtd. in Bray, 1962).

In a 1962 article, "The Effect of Egg Weight on Strain Differences in Embryonic and Postembryonic Growth in the Domestic Fowl," D.F. Bray found that, "egg size almost completely determined chick size and [the effect of egg size on chick size] rapidly decreased as the chicks became older (Bray, 1962)." Another article from 1962 by Bray and Iton and a supporting article in 1964 by Coleman *et al.* again found egg weight "to significantly affect embryo weight (Zervas and Collins, 1964)." In an article entitled,

"The influence of egg size on initial chick weight and subsequent broiler growth," Kh. Bondari and R. Kazemi again conclude, "the results of day-old body weight . . . indicated that a significant difference exists between the chicks hatched from different egg sizes. Large eggs hatched into larger chicks whereas smaller eggs resulted in smaller chicks (Bondari and Kazemi, 1975)." Bondari and Kazemi also found that the impact of egg size on body weight ceases after six weeks of age.

Albumen Removal:

From 1959 to 1975, Yutaka Mizuma and colleagues in the Department of Animal Husbandry at Tohoku University in Sendai, Japan, conducted a series of experiments entitled, *Studies On The Effect By The Embryonic Environment On The Characters of Chicken*. These trials examined the effects of albumen exchange, intergeneric albumen exchange, and albumen removal, on factors such as organ weight, metatarsal bone length, chick weight and growth, laying performance, embryonic growth, and hatchability in chickens.

Mizuma's first study focused on the development of a technique for albumen withdrawal and the optimal embryonic age at which to perform albumen removal experiments (Mizuma, I. "Influence of Albumen Exchange in Poultry Egg on Hatchability," 1959). Mizuma rules out other methods for albumen extraction proposed by G.A. Mastaler (1950) and L.R. Kuzunecov (1953) and opts for a method similar to the one used by Louisiana State University in the present experiment. Mizuma's method involves making a hole in the shell, directions for properly inserting the needle, and a plan for resealing the hole in the egg before further incubation (Mizuma, 1959). Mizuma

also concludes in his first study that, "the hatchability of the operated eggs are considerably influenced by the time of operation (Mizuma, 1959)."

In a second trial Mizuma is joined by Hisao Hashmia. This experiment involves the removal of 2.5, 5.0, 7.5, 10.0 and 12.5 mL from an average egg weighing 50g, amounts equivalent to 8.3, 16.6, 33.3, and 41.5 percent of the total albumen contained in the egg (Arai, Mizuma, and Nishida, 1971). In this trial, the removal of egg albumen was done after 24 hours of incubation and it results indicated that as more albumen was removed, hatchability became lower (Arai, Mizuma, and Nishida, 1971). Their study showed that up to 7.5 mL of albumen could be removed without affecting hatchability, that hatching was still possible when up to 10 mL of albumen were removed, and that removing 12.5 mL resulted in zero hatchability (Arai, Mizuma, and Nishida, 1971). This study also revealed a correlation between albumen removal and chick weight. Mizuma and Hashmia found, "that the decrease in egg-weight under the albumen removal treatment approximately corresponds to the quantity of the albumen removed (Arai, Mizuma, and Nishida, 1971, 227)." They go on to attribute this finding to the nutritious imbalance in the egg resulting from the removal of the albumen (Arai, Mizuma, and Nishida, 1971, 236).

In a fourth paper, Kanta Arai, Yutaka Mizuma, and Shusaku Nishida change their methods for operating on eggs. In this trial, the material used to seal their operated eggs changes from plaster (Mizuma, 1959) to a substance they call gyps (Arai, Mizuma, and Nishida, 1973). This particular trial finds a lower level of hatchability, although not significantly lower, in the experimental group than in the control group suggesting that the embryos were affected by the physical shock of the operation, but the declines in

hatchability could not be ensured statistically (Arai, Mizuma, and Nishida, 1973). This study also finds that chick hatch weight decreases proportionally to the amount of albumen removed (Arai, Mizuma, and Nishida, 1973) and that hatching weight can be highly correlated with egg size (Arai, Mizuma, and Nishida, 1971).

In a fifth trial, "The Effect of Albumen Removal on Embryonic Growth in Chickens," Kanta Arai, Yutaka Mizuma, and Shusaku Nishida tested the effect of the removal of 7.5 mL of albumen extracted after 72 hours of incubation. The researchers discovered significant differences in embryo weight between experimental and control groups at three points after 16 days of incubation (Arai, Mizuma, and Nishida, 1975) and also found highly significant differences in chick weight as a percentage of initial egg weight between the control group and the experimental group (Arai, Mizuma, and Nishida, 1975).

All previous research on albumen removal has observed the effects of the removal of large quantities of albumen (at least 7.5 mL) taken from eggs after some amount of incubation. Over the course of two trials, this experiment attempts to further examine the relationship between egg weight and chick weight, albumen removal, and its effects on hatchability and the ratio of chick weight to egg weight. The effects of this experiment are observed after the removal of far less albumen (maximum of 3 mL) from eggs prior to incubation.

Materials and Methods

Trial One:

Experimental Design

The conclusions of this trial reflect the results of two similar experiments. In the first experiment 300 fresh Cobb Broiler Breeder eggs were numbered and weighed to the nearest 0.1 gram on a Mettler scale (model BD601). The eggs were then divided into treatment groups of 75 eggs with each treatment further broken down into three replications of 25 eggs. The four treatment groups for the first experiment consisted of two controls, one, a control with no hole made and no albumen removed and a second control (sham) with a hole made, a needle inserted, but no albumen removed. In the third treatment 1 mL of albumen was removed and in the fourth treatment 2 mL of albumen were removed.

Albumen Removal

For all of the treatment groups except for the control, a hand engraver was used to remove a pin-point sized portion of the egg shell on the small end of the egg. The egg was then held upright as a 5 mL syringe with a 20 gauge, 1 ½ inch needle was inserted upward into the albumen through the weakened portion of the shell. Care was taken not to puncture the yolk or to damage the embryo. Once the needle was inside the egg, zero, one or two mL of albumen were extracted from the egg and discarded. Next, hot paraffin was applied over the hole in the egg shell with a syringe to seal the egg for incubation. Care was taken not to cover any more of the shell's pores with wax than necessary.

Incubation and Candling

The eggs were then placed in numerical order in setting trays and placed in a Natureform model NOM-45 incubator at 37.5 degrees Centigrade and 60 % humidity. After seven days of incubation the setting trays were removed and the eggs were candled with a hand-held candling device. At this time, all infertile and dead embryos were removed from the setting trays and then broken out to confirm infertility. After candling, the eggs were returned to the incubator until the 18th day when they were transferred into pedigree baskets for hatch. Each egg was hand placed, in numerical order, into the pedigree baskets with a divider between each egg. Then the baskets were placed in the 36.9 degree Centigrade and 75 % humidity Natureform NOM-45 hatcher for the remaining four days of incubation.

Hatch, Records and Data Computation

After 21 days of incubation the chicks were removed from the hatcher and weighed individually. A record was kept of the egg number, chick weight, and, when an egg remained unhatched, of the presence of an intact egg or a pip.

Spreadsheets were created noting egg number, initial egg weight, presence or absence of hatched chick, and chick weight. Statistical analysis was performed using the Statistical Analysis System (SAS) using a randomized block design. An analysis of variance (ANOVA) was done at a probability level of .05 to determine significance and means were differentiated by a Duncan's Multiple Range test.

Trial 2:

The second trial was nearly identical to the first diverging in method only in the division of treatment groups. In Trial 2, 360 Broiler Breeder eggs were numbered, weighed, and recorded according to egg number and egg weight in grams. The eggs were then divided into four treatment groups of 90 eggs. One treatment was a control that was drilled and injected, but had no albumen removed. There was also a 1 mL removal group, a 2 mL removal group, and a group that had 3 mL of albumen removed. These four treatment groups were divided into replications, this time with 30 eggs each.

Setting, incubation, candling, and transfer procedures for Trial 2 were all identical to those in Trial 1. Records of number of eggs remaining after 21 days of incubation, number of pips, and chick weights were taken and tables of data with the same information as in Trial 1 underwent statistical analysis (ANOVA and Duncan's Range, $P<0.05$) to determine significance.

Results

Trial 1:

Mean egg weight for the non-drilled control treatment in Trial 1 was 53.9 g, 53.9g in the drilled control group, 54.5 g in the one mL removal group, and 55.0 g in the two mL removal group. Mean chick weights for these same groups were 36.9 g, 37.2 g, 36.5 g, and 36.2 g respectively (Table 1). After statistical analysis, egg weight was found not to be significantly different among the treatment groups and it was shown that chick weight was also not significantly affected by treatment.

A comparison of the ratio of chick weight to egg weight did show significant differences however. The non-drilled control group had an egg to chick weight ratio of .6849, the drilled control had a ratio of .6895, the 1 mL group a ratio of .6693, and the 2 mL group had an egg to chick weight ratio of .6577. Statistical analysis indicated that the chick to egg weight ratio of the two control groups were not significantly different, but that the 1 mL removal group was significantly different from the controls and the 2 mL removal group was both significantly different from the controls and from the 1 mL removal group (Table 1).

Table 1. Mean egg weight, chick weight, and the ratio of egg to chick weight of broiler breeder eggs as affected by albumen removal prior to incubation (Trial 1).

Treatment	Mean Egg Weight	Mean Chick Weight	Egg to Chick Weight Ratio
Non-Drilled Control, 0 mL Removed	53.9 A	36.9 A	.6849 A
Drilled Control, 0 mL Removed	53.9 A	37.2 A	.6895 A
1 mL Removed	54.5 A	36.5 A	.6693 B
2 mL Removed	55.0 A	36.2 A	.6579 C
P>F	.4240	.5290	.0001

A, B, C means with different letters are significantly different at P < .05

As shown in Table 2, the statistical analysis of the fertility data collected on the four treatment groups revealed no significant difference in the percent of pips, the percent of intact eggs, percent hatch, percent fertile hatch, or percent fertility. These findings suggest that while the removal of 1 and 2 mL of albumen does affect the ratio of chick weight to egg weight, it does not affect fertility or hatchability levels.

Table 2. The percentage of pips, intact eggs, fertile eggs, total hatch, and fertile hatch for broiler breeder eggs as affected by albumen removal prior to incubation (Trial 1).

Treatment	% Fertile	% Pip	% Unhatched Egg	% Fertile Hatch	% Total Hatch
Non-Drilled, 0 mL Removed	89.3	2.7	6.7	89.6	80.0
Drilled, 0 mL Removed	66.3	0.0	10.7	76.6	55.6
1 mL Removed	58.7	1.3	12.0	72.7	45.3
2 mL Removed	52.0	0.0	8.0	85.9	44.0
P>F	.25	.84	.78	.78	.38

Trial 2:

Trial 2 of this experiment used only one control treatment that had its shell drilled, a syringe inserted, but no albumen removed. The three other treatment groups consisted of eggs with 1, 2, and 3 mL of albumen removed. As in Trial 1, this trial showed no significant correlation between albumen withdrawal and hatchability. But again, a link was discovered between albumen extraction and the ratio of chick weight to egg weight.

As in the first trial, albumen withdrawal had no effect on egg weight or chick weight, but a significant relationship was seen between albumen removal and the ratio between egg weight and chick weight. The numerical values for mean egg weight,

mean chick weight and the mean egg to chick weight ratios for the four treatments can be found in Table 3.

Table 3. Mean egg weight, chick weight, and the ratio of egg to chick weight for broiler breeder eggs as affected by albumen removal prior to incubation (Trial 2).

Treatment	Mean Egg Weight	Mean Chick Weight	Ratio
0 mL	65.4 A	45.3 A	.6917 A
1 mL	67.7 A	44.7 A	.6594 AB
2 mL	67.7 A	45.1 A	.6657 B
3 mL	67.3 A	43.5 A	.6457 B
P>F	.55	.81	.04

A, B means with different letters are significantly different at P < .05

As Table 4 shows, after statistical analysis all the P values for the hatch data were above the .05 significance threshold showing that in addition to having no affect on hatchability, albumen removal had no affect on the percentage of pips recorded, the percentage of unhatched eggs, or the overall percentage of eggs that hatched.

Table 4. The percent fertility, unhatched eggs, pips, fertile hatch and total hatch (Trial 2).

Treatment	% Fertility	% Pips	% Unhatched Eggs	% Fertile Hatch	% Total Hatc
0 mL	44.4	2.2	25.6	38.9	16.7
1 mL	60.0	7.8	28.9	37.3	23.3
2 mL	57.8	5.6	26.7	47.2	25.6
3 mL	52.2	5.6	32.2	27.1	14.4
P>F	.67	.33	.88	.32	.34

Discussion

The results of this experiment support the findings of Mizuma and his colleagues. In their second paper, Mizuma, Aria, and Nishida showed no deleterious effects of albumen removal on hatchability when 7.5 mL or less albumen was removed (Aria, et. al II). In both trials of this experiment, albumen removal proved to have no effect on hatchability. The two types of controls in the first trial indicated that the physical stresses of the experimental method itself did not significantly harm the ability of an embryo to develop and hatch and that the removal of two mL of albumen also had no negative effect on hatchability. In Trial 2, 3 mL of albumen were removed, and again no recorded effect on hatchability was observed.

This experiment supports earlier findings on the relationship between egg weight and chick weight as well. The importance of albumen in embryo development and nutrition is well understood. The correlation between egg weight and chick weight has been studied since the early days of poultry research and is well documented. Halbersleben and Mussehl, Upp, Jull and Heywang, Wiley, D.F. Bray, Iton, and Kh. Bondari and R. Kazemi have all observed a direct effect of egg weight on the weight of hatched chicks (Bray Coleman, Zervas, Bondari). In both trials of this experiment eggs were not specially selected for weight and yet no significant difference in weight was recorded among them. This homogeneity guarded the experiment against the sited effects of egg size on chick size; because no difference was found among egg weights it would suggest that differences in chick weight could be attributed to experimental procedures. The results of this experiment also indicate that albumen removal has no effect on chick weight when chick weight alone is examined. When compared to the

original weight of the egg however, both Trials 1 and 2 of this experiment showed a significant difference in the ratio of chick weight to egg weight between the treatment groups and the control group. The results show that as little as 1 mL of albumen removed from the egg will produce chicks with a lower body weight relative to their weight as eggs. Because of the paucity of variance, when measured as a direct comparison of individual chick weight to egg weight no change can be detected, but when analyzed by treatment groups of multiple eggs, a significant decline in chick weight when compared to original egg weight can be seen.

Along with previous studies of the effect of albumen withdrawal on hatchability, this experiment shows that no negative effect on hatchability will result when up to 3 mL of albumen are removed from a fertilized egg. These findings endorse the work of researchers manipulating the amounts of albumen in eggs in order to perform tests on the egg and on the nascent embryo even though the removal of albumen in this experiment was removed prior to incubation. At least 3 mL of albumen and perhaps as many as 7.5 mL of albumen can be removed from an egg without affecting hatchability, but these trials, like others before them, do show an effect on the ratio of chick weight to egg weight in treatment groups that have had albumen removed.

Wiley, in 1950, and Bondari and Kazemi in 1975 showed that the effect of egg size on chick weight dissipates after several weeks of growth. Further work should be done to investigate the long-term effects of albumen removal on poultry. While this experiment shows no effect on hatchability, long-term problems could surface because of albumen removal procedures. To test the full effects of albumen removal on poultry, additional egg weight data could be collected after albumen withdrawal to clarify the

effects of egg weight on chick weight, and the chicks hatching in albumen extraction studies could be monitored for body weight and feed conversion rates over the growing period.

These results are promising, but if future experimentation shows that broilers require greater than six weeks to recover body weight lost because of albumen removal, then any prenatal alterations in the egg, no matter how beneficial, may prove to be unimportant to the profit-driven poultry industry. Because bird weight at market determines earnings, the benefits of improvements in birds as a result of research centered on albumen removal may be lost if body weight cannot be successfully regained before slaughter.

Summary and Conclusion

All eggs used in this experiment proved to be of relatively equal size despite their random selection. The chicks hatching from the eggs in this experiment also showed no significant difference in body weight. These two findings helped to isolate the effects of albumen removal on the developing embryo. The results show that the removal of up to 3 mL of albumen has no effect on hatchability, but that albumen withdrawal does significantly change the ratio of chick weight to egg weight. This experiment supports previous findings on the safety of albumen removal for hatchability, but suggests that albumen extraction is done at the cost of chick body weight at hatch. Because the process of albumen removal itself seems to have no negative effects on hatchability when up to 3 mL of albumen are removed, researchers are free to conduct other studies requiring albumen removal so long as they accept the inevitability of an effect on the relationship between the weight of the eggs they used and the size of the chicks they are hatching as a result of albumen removal.

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