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CALCIUM DYNAMICS AFFECTING EGG PRODUCTION,
SKELETAL INTEGRITY, AND EGG COLORATION
IN RING-NECKED PHEASANTS
(PHASIANUS COLCHICUS)

by

Landon R. Jones

A thesis submitted to the faculty of

Brigham Young University

in partial fulfillment of the requirements for the degree of

Master of Science

Department of Plant and Wildlife Sciences

Brigham Young University

December 2007

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BRIGHAM YOUNG UNIVERSITY

GRADUATE COMMITTEE APPROVAL

of a thesis submitted by

Landon R. Jones

This thesis has been read by each member of the following graduate committee and by majority vote has been found to be satisfactory.

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BRIGHAM YOUNG UNIVERSITY

As chair of the candidate's graduate committee, I have read the dissertation of Landon R. Jones in its final form and have found that (1) its format, citations, and bibliographic style are consistent and acceptable and fulfill university and department style requirements; (2) its illustrative materials including figures, tables, and charts are in place; and (3) the final manuscript is satisfactory to the graduate committee and is ready for submission to the university library.

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ABSTRACT

CALCIUM DYNAMICS AFFECTING EGG PRODUCTION, SKELETAL INTEGRITY, AND EGG COLORATION IN RING-NECKED PHEASANTS (*PHASIANUS COLCHICUS*)

Landon R. Jones

Department of Plant and Wildlife Sciences

Master of Science

These 4 chapters represent manuscripts formatted for submission to journals based on an experiment conducted on Ring-necked Pheasants (*Phasianus colchicus*). Calcium limits the distribution of this species, which produces 7-15 eggs per clutch. They may reneest up to 5 times per breeding season on a diet low in calcium. The first chapter examines egg production in laying hens on 7 different diets from 0.19-4.47% calcium in the absence of calcium loading. Calcium-loaded pheasants store calcium in medullary bone before an experiment and can draw on this surplus during egg production, possibly skewing experimental results. We measured egg production, egg characteristics, and hen femur ash fraction in a 2-month experiment. Hens in the middle 5 dietary calcium (1.07-3.08%) groups statistically produced the same number of eggs, which differed from reported studies where hens were calcium-loaded. Ash fraction values indicated that hens expended medullary bone reserves to produce eggs when dietary input was low. In

Chapter 2, we examined bone properties in the above femurs to determine if pheasant hens on low calcium diets expended enough medullary bone stores to compromise skeletal integrity. We applied a 3-point bending test to find femur breaking strength and examined structural bone properties. Calcium and breaking strength were linearly associated. Femurs of hens given lower calcium diets were easier to break. Structural properties of cortical bone were not correlated with dietary calcium. Pheasants on low calcium, comparable to wild conditions, seemed to sacrifice skeletal integrity to maintain high egg production, although not enough to damage cortical bone. In Chapter 3 we examined 437 eggs laid to determine if egg color correlated with dietary calcium, egg mass, volume or shell thickness. Yellow pigment decreased with increasing calcium. Biliverdin had a higher affinity for calcium than protoporphyrin. In Chapter 4, we examined male pheasants to determine if reduced surface area on one wing (clipped) induced unbalanced pectoralis muscle development and humerus density on the corresponding side after wing-whirring for 2 months. We weighed pectoralis muscles and conducted 3-point bending tests on humeri of 7 pheasant males. We found no difference in pectoralis muscles mass, humeri breaking strength or ash fraction between clipped and unclipped wings. Wing-whirring may only put a negligible strain on male Ring-necked Pheasant pectoralis muscles and humeri.

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Effects of calcium loading on egg production in Ring-necked Pheasant (*Phasianus colchicus*) hens.

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Abstract

Calcium limits the distribution of the Ring-necked Pheasant (*Phasianus colchicus*) in the U.S. Experiments reportedly conducted to measure pheasant egg production with varying levels of dietary calcium probably did not account for calcium loading before applying treatment diets. Calcium-loaded pheasants store calcium in medullary bone before the experiment and can draw on the surplus during egg production, possibly skewing experimental results. We measured egg production, egg characteristics, and femur ash fraction for pheasants on 7 diets differing in calcium percent from 0.19-4.47% without calcium loading. This condition more closely mimics the wild. Hens in the middle 5 calcium groups produced the same number of eggs statistically, which differed from literature reports. Because pheasant hens only live up to 3 years in the wild, we expected them to put more energy into reproduction than health condition, expending medullary bone reserves to produce eggs when dietary input is low. Egg production and ash fraction

results supported this expectation. Mass, volume, and shell thickness were not significantly different among calcium groups, consistent with the literature.

Introduction

The Ring-necked Pheasant (*Phasianus colchicus*) is a popular game bird introduced from Eurasia to the U.S, where it is widely distributed. (Giudice and Ratti 2001). Hens can produce 7-15 eggs per clutch and reneest up to 5 times per season on a granivorous diet low in calcium (Giudice and Ratti 2001). Leopold (1931) noticed that Ring-necked Pheasants released on glacial soil deposits rich in calcium, such as in the North-Central states, seemed to persist whereas pheasant introductions on unglaciated deposits, apparently calcium-poor, persisted a few years, but eventually failed. He theorized that calcium was a basic dietary requirement for pheasants and that future introductions would thrive on calcium-rich soils. Many authors have since suggested that calcium may be a limiting factor for the distribution of pheasants in the U.S., especially in the Midwestern Corn Belt (Dalke 1938, McCann 1939, Gerstell 1937, Dale 1954, 1955, Dale and DeWitt 1958, Korschgen 1961, Sadler 1961, Harper 1964, Harper and Labisky 1964). Several studies have also determined the optimum dietary calcium requirement to estimate wild population laying dynamics or maintain laying hens at commercial game farms (Greeley 1962, Chambers et al. 1966, Hinkson et al. 1970, Wise and Ewins 1980, Reddy and Reddy 1985).

Greeley (1962) experimentally maintained pheasant hens on 5 different calcium diets: 0.37%, 0.63%, 1.09%, 2.01% and 2.34%. He found reduced egg production, reduced femur ash and eggshell thickness at the 1.09% level and lower. Chambers et al.

(1966) kept pheasants on calcium diets of approximately 0.5%, 0.8%, 1.5% and 3.2% and found the highest egg production at the 3.2% level. Hinkson et al. (1970) found the highest egg production on a calcium diet of 2.5% out of the following levels: 0.9%, 1.8%, 2.5% and 3.7%. Wise and Ewins (1980) gave pheasant hens calcium diets of 2.0%, 2.3%, 2.3% plus grit, and 2.7%. They found no difference in egg production among any of the 4 groups. Reddy and Reddy (1985) examined dietary calcium levels of 0.94%, 2.50% and 4.00% and found superior egg production at the 2.50% level. The National Research Council (1994) suggests that 2.5% calcium is optimal.

The above data seem to indicate that the optimum dietary calcium level for egg production, at least for captive pheasants in a game farm scenario, ranges from 2.0% to 3.2%. Any of these percentages may yield maximum egg production if additional nutrients are balanced appropriately. However, some of these results may be due to calcium loading before the experiment. Calcium loading occurs when birds are given a high calcium diet prior to egg laying and are allowed time to accumulate calcium in medullary bone for later egg production. Pheasant hens in the above experiments were either given normal laying mash of up to 3% dietary calcium, or didn't report their pre-experimental diets before switching hens to treatment diets.

Birds are able to store calcium as medullary bone, a motile bone layer that forms due to increased estrogen levels in preparation for the breeding season (Pfeiffer and Gardner 1938). These calcium reserves, along with cortical bone, can serve as a buffer to calcium needs during egg production (Urist 1959). Pheasants that have been calcium-loaded before egg production will be able to expend skeletal reserves when dietary

calcium is lacking. We predicted that the number of eggs calcium-loaded hens produced should over-estimate the actual numbers produced on dietary calcium alone.

Methods

We obtained 48 pheasants, 42 yearling hens and 6 males, from a game farm in Mona, Utah on 16 February 2007. These pheasants were probably several generations removed from the wild. The hens were weighed and randomly assigned to 7- 10'x10'x10' wire pens with raised, plywood floors. One male was also arbitrarily placed in each pen after weighing according to Greeley (1962) and Reddy and Reddy (1985). Pheasants were given a ration of Leland Mills (Spanish Fork, UT) commercial feed diet containing 0.19% calcium at the game farm throughout the winter and also had access to soils. They were given the same ration for 3 weeks after arrival at our site but had no access to soils. After this 3-week acclimation period, we switched 6 of the 7 diets, differing in calcium percentages: 1.07%, 1.68%, 2.05%, 2.84%, 3.08% and 4.47%. We mixed calcite containing 32% calcium into the commercial mix evenly to create each treatment diet. The ingredients and nutrient contents of the commercial and experimental diets are shown in Table 1. All diets satisfied nutritional requirements for laying Ring-necked Pheasant hens according to the National Research Council (1994). One pen was maintained on the 0.19% diet as a control. We rotated males weekly to adjacent pens to ensure males had a uniform effect on fertility among the pens (Hinkson et al. 1970, Reddy and Reddy 1985).

We collected eggs daily from each pen from 10 March to 9 May 2007. We measured the mass of each egg to the nearest 0.1 g on an electronic balance and the

volume to the nearest 0.5 ml in a graduated cylinder by water displacement. We measured the shell thickness of each egg with vernier calipers to the nearest 0.001 mm, after cutting each egg open lengthwise with a dental drill and removing the shell membrane, following Greeley's (1962) method. We took the average of 2 measurements at each end of the egg and at its midpoint and averaged all 3 measurements to obtain the overall shell thickness for each egg. On 11 May, we sacrificed the pheasants, weighed them again and removed the right and left femurs of all hens. Femur ash fraction was determined at the Donahue Laboratory at Michigan Technological University, Houghton, MI. We conducted multivariate statistical analyses in SAS Version 9.1 (SAS Institute Inc., Cary, NC) to assess differences in egg production, egg mass, egg volume, shell thickness, hen weight loss percentage, and femur ash fraction between calcium groups.

Results

The percent of hens laying per day, number of eggs produced, average egg volume, mass, and shell thickness of eggs from each dietary calcium group are shown in Table 2. The 1.07% calcium group produced the highest number of eggs ($n=88$, Fig. 1), although this number did not differ statistically from those produced in the 1.68%, 2.05%, 2.84%, or 3.08% dietary calcium groups. The lowest level of egg production was the 0.19% calcium diet ($n=12$), which differed significantly ($p<0.05$) from every other diet except 4.47% ($n=39$, Fig. 1). Average egg volume, mass and shell thickness did not differ statistically between groups.

Average hen weights for each calcium group before and after the experiment are shown in Table 3. The 2.05% and 4.47% calcium diet groups differed significantly from

the other groups in percent body weight lost, but a linear regression test showed no correlation between hen weight loss and dietary calcium percent ($p=0.096$). Only 1 hen gained weight (0.19% group), the others lost weight. The average femur ash fraction for hens in each dietary group is shown in Figure 2. Ash fraction data showed a 2nd degree polynomial fit ($p=0.010$) when plotted against dietary calcium percent (Fig. 2).

Discussion

Our egg production results differed from those reported in the literature. Greeley (1962), Hinkson et al. (1970), and Reddy and Reddy (1985), all found maximum egg production in hens given a calcium diet of 2.34-2.50%. Egg production also decreased below or above these levels. Wise and Ewins (1979) found no difference between egg production in pheasant hens given calcium diets between 2.2-2.8%. The results of Chambers et al. (1966) found the highest egg production mainly at the 3.2% calcium level. Our results showed that pheasant hen egg production was the same at 5 of our 7 dietary calcium levels: 1.07%, 1.68%, 2.05%, 2.84%, or 3.08% (Fig. 1).

Our study compared best with Greeley (1962), since our sample sizes and experimental designs were similar. Both experiments were conducted for approximately 2 months and both experiments relied on natural sunlight to induce egg production during the normal breeding season. Greeley's hens were calcium-loaded on a 3% diet for 3 weeks before the experiment began. Our hens were only given a 0.19% calcium diet for the same time period and had also been given the same diet all winter at the game farm, but had access to soils. Table 4 compares our egg production results to those of Greeley. Where Greeley used an additional replication, 10 groups of 6 hens at 5 calcium levels

($n=60$), we added 2 treatment levels with the same number of hens in each calcium group ($n=48$). At the most depressed dietary calcium level (0.37%), Greeley's hens averaged 10.5 eggs between 2 replications. Our hens, on the lowest level (0.19% calcium), performed similarly, producing 12 total eggs. At the 0.63% and 1.09% levels, Greeley's hens still produced few eggs (27.5 and 34.5 respectively), whereas our hens produced the highest number of eggs ($n=70-88$) at the middle 5 levels, which did not differ statistically from each other. Greeley's hens did not reach peak production until the 2.01% and 2.34% calcium levels.

The discrepancies between our egg production and Greeley's may be explained by calcium loading. However, some of our results seem counterintuitive. We predicted that our hens, which were not calcium-loaded, should produce fewer eggs on the same calcium diets than those in other studies. Our hens produced fewer eggs at the 2.05% and 2.84% levels ($n=85$ and 73 respectively) than Greeley's hens at the 2.01% and 2.34% levels ($n=100.5$ and 121). However, our hens at the lower calcium levels, not to include the lowest level in each study (0.37% and 0.19%), produced significantly more eggs. At the 1.07% and 1.68% levels, our hens produced 88 and 78 eggs versus the average of 34.5 eggs that Greeley's hens produced on a 1.09% calcium diet. These data do not support our predictions and warrant explanation.

Since Ring-necked Pheasant hens survive an average of 3 years in the wild, or 3 possible breeding seasons, they must make the most out of each reproductive event. As ground nesters, they must produce many more eggs in the breeding season than most birds to ensure their same few offspring survive. From an evolutionary perspective, hen pheasants should produce as many eggs as possible during any given breeding season,

even at the expense of their own health because the probability of their surviving to the next breeding season is low. Hens that have stored calcium in medullary bone in preparation for the breeding season, even with low dietary calcium input throughout the season, may have enough calcium to balance eggshell production needs with bone stores without much stress or health reduction. This could explain the low egg production results Greeley obtained at the 1.09% calcium level with calcium-loaded hens. Those hens were not calcium-stressed, but produced fewer eggs ($n=34$). They seemed to strike an adequate compromise between producing several eggs and maintaining health for the next breeding season when calcium might be more available.

On the other hand, a calcium-stressed hen, with little calcium available in medullary bone, may sacrifice future health for current egg production by using medullary bone stores. This mechanism would explain our results at the 1.07% and 1.68% calcium level. As seen in the 0.19% calcium diet and in Greeley's 0.37% diet, pheasant hens only produced an average of 2 eggs during the 2-month experimental period. Apparently, there was not enough calcium to elevate estrogen sufficient for sustained ovulation and egg production. However, at least 1.07% dietary calcium seems adequate to commence egg production and bone stores are able to maintain high production at the cost of future health.

Egg production may also be an artifact of a small sample size or genetic factors. Game farmers may have selected for more efficient egg-laying pheasant hens. Their capacity for calcium absorption and egg-laying may have been augmented genetically through breeder selection since the 1960s, when Greeley conducted his experiment. This may explain our results at the 1.07 and 1.68% levels, but egg production at the higher

levels underestimated Greeley's (1962) results, as we predicted for calcium loading. Although game farm pheasants may be many generations removed from the wild, they have not been subjected to the same intense selective pressures as domestic chickens.

We found that the average egg volume, mass and shell thickness did not differ statistically between dietary calcium groups. These results are similar to Greeley (1962), Chambers et al. (1966), Hinkson et al. (1970), Wise and Ewins (1979), and Reddy and Reddy (1985). Because egg production is a costly endeavor in terms of energy and probably under evolutionary selection, pheasant hens could either produce eggs that have a high chance of hatching or forgo egg production entirely. Any hens that produced eggs with a mass, volume or shell thickness outside of this viable range would be selected against in the wild.

All pheasant hens except 1 lost weight during the experiment, which is typical during the laying season (Kabat et al. 1950, 1956). The hen that gained weight came from the 0.19% calcium group that produced very few eggs. We were unable to sequester individual hens and cannot say for certain whether this hen produced any eggs. The hens in the 0.19% calcium group probably did not have enough calcium feedback in their diet to stimulate reproductive activity (Sturkie 1954). Although we found statistical differences in 2 calcium groups (2.05% and 4.47%), we found no lineal trend when we compared hen weight loss with calcium group ($p=0.096$). These findings parallel those of Greeley (1962).

Calcium loading may also explain why our ash fraction results (Fig. 2) mirrored egg production and followed a polynomial trend. Dietary calcium levels above approximately 3.2% seemed to depress calcium availability for egg production and

calcium for bone storage (Hinkson et al. 1970, Reddy and Reddy 1985, Fig. 1, Fig. 2). During Greeley's (1962) 2-month experiment, each group started out with the same "saturated" amount of calcium in their bone stores, but had to draw from it according to dietary calcium input, accounting for the linear ash fraction trend and increased calcium levels. The experiments of Chambers et al. (1966), Hinkson et al. (1970) and Reddy and Reddy (1985), who found no ash fraction trend, ran for several months, which may have given hens time to replenish bone stores enough to invalidate the linear trend Greeley (1962) found. Differences in the experimental designs among studies may also account for their ash fraction results. Because we ran our experiment for approximately the same amount of time and in similar conditions as Greeley (1962), calcium loading seems to be the only variable that can account for reduced ash fraction at the higher calcium levels.

Calcium loading in captive birds that were introduced on calcium-poor soils in the early 1900s (Leopold 1931) probably accounts for the persistence of pheasants over a few breeding seasons, followed by their eventual extirpation.

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Table 1. Ingredients and nutrient contents of commercial and experimental diets given to 7 laying hen pheasant groups.

| Ingredient | Diets g/100 g | | | | | | |
|---------------------|----------------------|------------|------------|------------|------------|------------|------------|
| | Control | 1 | 2 | 3 | 4 | 5 | 6 |
| Corn, yellow ground | 65.0 | 62.4 | 60.6 | 59.5 | 57.2 | 56.5 | 52.4 |
| Soybean meal (44%) | 27.5 | 27.5 | 27.5 | 27.5 | 27.5 | 27.5 | 27.5 |
| Wheat bran | 6.25 | 6.25 | 6.25 | 6.25 | 6.25 | 6.25 | 6.25 |
| Salt | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Vitamin premix* | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Dicalcium phosphate | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Calcite | 0.0 | 2.6 | 4.4 | 5.5 | 7.8 | 8.5 | 12.6 |
| Total | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Analysis | | | | | | | |
| Protein | 19.2 | 19.0 | 18.8 | 18.7 | 18.5 | 18.4 | 18.0 |
| Methionine-Cystine | 0.61 | 0.60 | 0.59 | 0.59 | 0.58 | 0.58 | 0.56 |
| Lysine | 1.01 | 1.00 | 0.99 | 0.99 | 0.98 | 0.98 | 0.97 |
| Calcium | 0.19 | 1.07 | 1.68 | 2.05 | 2.84 | 3.08 | 4.47 |
| Phosphorus | 0.47 | 0.47 | 0.46 | 0.46 | 0.46 | 0.45 | 0.45 |

*Premix supplies per kg of diet the following amounts: calcium 0.34 g, magnesium 0.01 g, phosphorus 0.01 g, potassium 0.01 g, copper 4.4 mg, iodine 2.27 mg, iron 38.13 mg, manganese 66.0 mg, selenium 0.10 mg, zinc 60.03 mg, vitamin A 6666.0 IU, vitamin D 3305.5 IU, vitamin E 8.26 IU, niacin 22.01 mg, riboflavin 3.85 mg, pantothenic acid 5.4 mg, folic acid 0.23 mg, choline 274.92 mg, vitamin B12 1.0 mcg, pyroxidine 1.09 mg, menadione 0.55 mg, thiamine 1.45 mg.

Table 2. Performance of pheasant hens on 7 different calcium diets, showing the percentage of hens that laid per day, number of eggs produced, and average egg volume, mass, and shell thickness per group.

| Dietary Calcium % | Production %Hen/Day | Eggs Produced | Avg Egg Volume (mL) | Avg Egg Mass (g) | Avg shell thickness (μ) |
|--------------------------|----------------------------|----------------------|----------------------------|-------------------------|---|
| 0.19 | 0.03 | 12 | 25.87 | 24.10 | 0.325 |
| 1.07 | 24.04 | 88 | 25.30 | 23.30 | 0.379 |
| 1.68 | 21.31 | 78 | 25.38 | 23.28 | 0.397 |
| 2.05 | 23.22 | 85 | 24.89 | 22.99 | 0.373 |
| 2.84 | 19.95 | 73 | 24.05 | 22.17 | 0.390 |
| 3.08 | 19.13 | 70 | 23.14 | 21.32 | 0.387 |
| 4.47 | 10.66 | 39 | 24.18 | 22.33 | 0.392 |

Table 3. Average percent body weight lost by each pheasant group from 10 March to 9 May 2007.

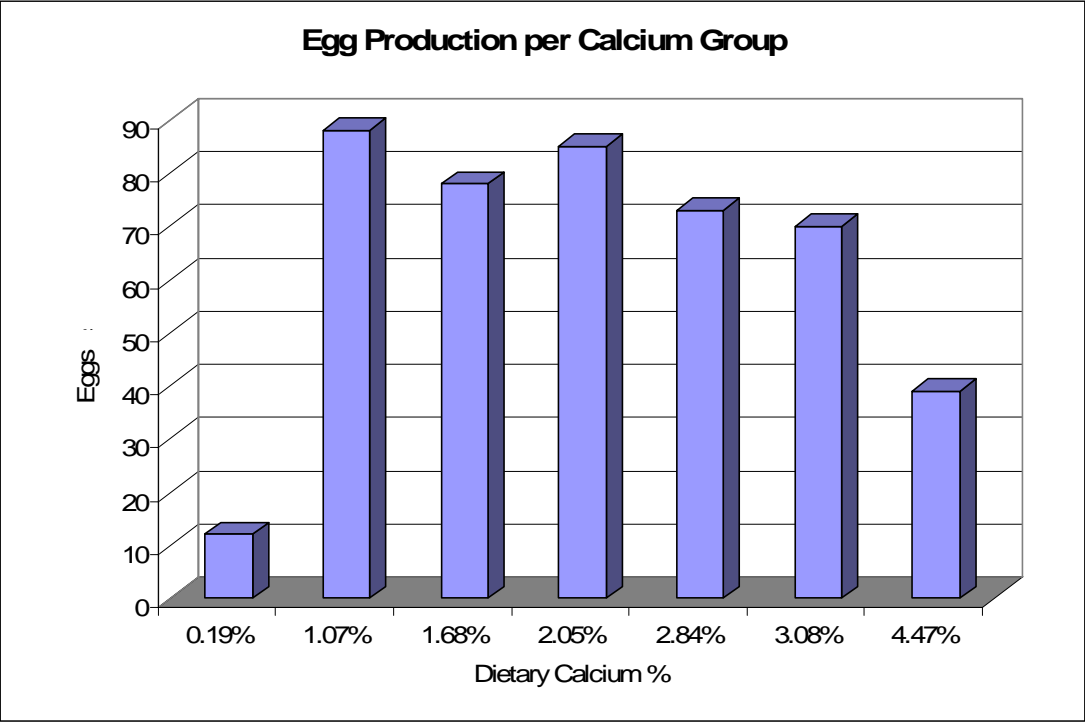
| Dietary Calcium % | Start Mass (g) | End Mass (g) | Average Body Weight Loss % |
|--------------------------|-----------------------|---------------------|-----------------------------------|
| 0.19 | 942.6 | 805.6 | -13.05 |
| 1.07 | 952.1 | 798.5 | -15.99 |
| 1.68 | 907.2 | 789.1 | -12.80 |
| 2.05 | 935.5 | 682.8 | -26.56 |
| 2.84 | 914.3 | 751.3 | -17.76 |
| 3.08 | 916.6 | 772.5 | -15.76 |
| 4.47 | 959.2 | 670.9 | -29.52 |

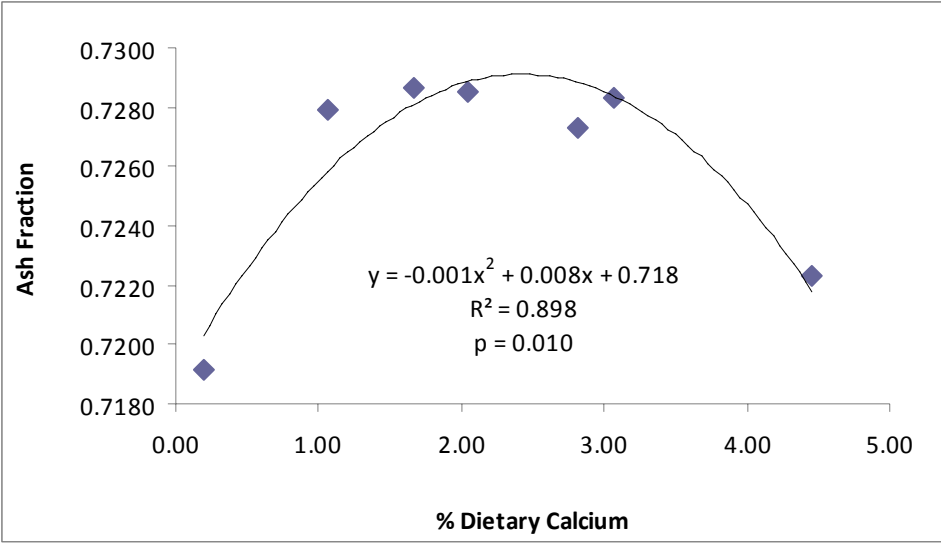
Table 4. Eggs produced for each calcium level between Greeley’s (1962) study and our study. Greeley had 2 replications, our study had 1.

| Greeley (1962) | | Jones et al. (2007) | |
|----------------------------|-----------------------------|--------------------------------|----------------------|
| Dietary calcium | Eggs per replication | Dietary calcium | Eggs produced |
| 0.37% | 10.5 | 0.19% | 12 |
| 0.63% | 27.5 | 1.07% | 88 |
| 1.09% | 34.5 | 1.68% | 78 |
| 2.01% | 100.5 | 2.05% | 85 |
| 2.34% | 121 | 2.84% | 73 |
| | | 3.08% | 70 |
| | | 4.47% | 39 |

Figure 1. Number of eggs produced by pheasant hens in 7 dietary calcium groups.

Figure 2. Average femur ash fraction (ash mass/dry mass) values for 7 hen pheasant dietary calcium groups.





RRH: *Jones et al.* • Ring-necked Pheasant calcium choices

Reproduce or survive: choices of Ring-necked Pheasant (*Phasianus colchicus*) hens in a calcium-deficient world.

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ABSTRACT.--- Calcium limits the distribution of the Ring-necked Pheasant (*Phasianus colchicus*) in the U.S. Birds store calcium as medullary bone for egg production needs during the breeding season. We examined egg production, ultimate load, ultimate stress, cross-sectional properties of cortical bone, and femur ash fraction in 42 hen pheasants given seven different calcium diets during an 8-week period. Hens on the middle five diets statistically produced the same number of eggs. Femurs of hens given lower calcium diets were easier to break and contained lower mineral content. Cross-sectional (structural) properties of cortical bone were not correlated with dietary calcium. Pheasants on low calcium diets, comparable to wild conditions, seemed to sacrifice skeletal integrity to maintain high egg production during the breeding season, but not enough to damage cortical bone.

Aldo Leopold (1931) first suggested that calcium may limit the distribution of the Ring-necked Pheasant (*Phasianus colchicus*), an introduced game bird from Eurasia, in the U.S. Many authors have since provided evidence to support this idea (Gerstell 1937, Dalke 1938, McCann 1939, Dale 1954 and 1955, Dale and DeWitt 1958, Korschgen 1961, Sadler 1961, Harper 1964, Harper and Labisky 1964). Ring-necked Pheasant hens are ground-nesting birds that can produce 7-15 eggs per clutch and renest up to five times per season on a granivorous diet low in calcium (Giudice and Ratti 2001). Because of this large calcium requirement, calcium-deficient soils pose a unique problem to pheasant survival.

Gallinaceous birds typically have long bones which allow them to maintain significant calcium stores in medullary bone, a motile bone layer that forms due to increased estrogen levels in preparation for the breeding season (Pfeiffer and Gardner 1938). For pheasants, these calcium reserves, along with cortical bone, can supply calcium during egg production when dietary calcium input is low (Urist 1959). However, if pheasant hens draw too heavily on bone stores for egg production, they may compromise skeletal integrity and negatively impact their own health. Several studies have shown that osteoporosis occurs in domestic chicken hens when calcium levels are insufficient in their laying ration (Wilson and Duff 1991, Rennie et al. 1997, Fleming et al. 1998b, Fleming et al. 2003, Schreiweis et al. 2003, Fleming et al 2006).

Chambers et al. (1966) experimentally examined the effects of different calcium diets on egg production and femur histological structure. They found enlarged Haversian canals, thinner femur walls, and osteoporosis in hens at the 0.5% calcium level. They also suggested that osteoporosis in wild hens may indicate insufficient available calcium in

the habitat. Egg production results for each calcium diet increased with dietary calcium.

Ring-necked Pheasants typically survive up to 3 years in the wild and yearlings hens breed their first spring (Giudice and Ratti 2001). Because pheasant hens, embryos and chicks have a high mortality rate, hens must lay as many eggs as possible to ensure some progeny survival. Based on their life history data, pheasant hens should trade future survival for current reproduction. We predicted that captive pheasant hens on low-calcium diets would produce as many eggs as hens given sufficient calcium for sustained egg production, but that the calcium-deficient hens would exhibit loss of skeletal integrity.

METHODS

We obtained 48 pheasants, 42 yearling hens and 6 males, from a game farm in Mona, Utah on 16 February 2007. These pheasants were probably several generations removed from the wild. The hens were weighed and randomly assigned to seven 10'x10'x10' wire pens with raised, plywood floors. One male was also arbitrarily assigned to each pen after weighing according to Greeley (1962) and Reddy and Reddy (1985). We randomly chose and sacrificed 6 pheasant hens and removed their femurs to assess 0-time bone mass. Pheasants were given a ration of Leland Mills (Spanish Fork, UT) commercial feed diet containing 0.19% calcium at the game farm throughout the winter and also had access to soils. They were given the same ration for 3 weeks after arrival at our site but had no access to soils in our experimental pens. The ingredients and nutrient contents of the commercial and experimental diets are shown in Table 1. After this 3-week acclimation period, we switched six of the seven diets to the experimental

regimes, differing only in calcium percentage: 1.07%, 1.68%, 2.05%, 2.84%, 3.08% and 4.47%. We mixed calcite containing 32% calcium into the commercial mix evenly to create each treatment diet. The ingredients and nutrient contents of the commercial and experimental diets are shown in Table 1. All diets satisfied nutritional requirements for laying Ring-necked Pheasant hens according to the National Research Council (1994). One pen was maintained on the 0.19% diet as a control. We rotated males weekly to adjacent pens to ensure males had a uniform effect on fertility among the pens according to Hinkson et al. (1970) and Reddy and Reddy (1985).

We collected eggs daily from each pen from 10 March to 9 May 2007. On 11 May, we sacrificed the pheasants and removed both femurs. Bone strength and porosity were determined at the Donahue Laboratory at Michigan Technological University, Houghton, MI. Ten mm bone segments extending distally from the femoral midshaft were removed from the left femurs, fixed in 70% ethanol, and embedded in methyl methacrylate. The midshaft cross-section was exposed with a diamond saw and imaged with a digital camera (Spot Insight QE, Diagnostic Instruments Inc., Sterling Heights, MI). Image analysis software (Scion Image, Frederick, MD; Bioquant Osteo, Nashville, TN) was used in conjunction with a custom macro to calculate bone cross-sectional properties including cortical area (Ct.Ar) and the cross-sectional moments of inertia for the mediolateral (I_{ML}) and anteroposterior (I_{AP}) axes.

Right femurs were loaded to failure in three-point bending on an Instron mechanical testing system (Model #8872, Canton, MA) at a rate of 10 mm/min. Bones were oriented with the anterior surface in tension on supports separated by a span of 55 mm, and all fixtures had rounded contact points (radius=6.4 mm). After testing, ultimate

load and ultimate stress were calculated using the load-deformation data from testing and the mediolateral moment of inertia for the left femoral midshaft described above.

10 mm bone segments extending proximally from the femoral midshaft were removed from the left femurs and cleaned of marrow. Bone segments were placed into a furnace at 100° C for 24 hr to remove water, weighed (dry mass), then placed into a furnace at 600° C for 48 hr to remove organic matrix, and weighed again (ash mass). Ash fraction, a measure of mineral content, was calculated as the ash mass divided by the dry mass.

Multivariate statistics were calculated for egg production differences between dietary calcium groups in SAS Version 9.1 (SAS Institute Inc., Cary, NC). Regression analyses for ultimate load, ultimate stress, cross-sectional properties, and femur ash fraction were conducted in Excel (Microsoft Corporation, Redmond, WA).

RESULTS

Egg production for each calcium group is shown in Figure 1. The 1.07% calcium group produced the highest number of eggs ($n=88$), although this number did not differ statistically from those produced in the 1.68%, 2.05%, 2.84%, or 3.08% dietary calcium groups. The lowest level of egg production was the 0.19% calcium diet ($n=12$), which differed significantly ($p<0.05$) from other diets except 4.47% ($n=39$).

Ultimate load was linearly associated with increased dietary calcium ($p=0.005$, Fig. 2). Ultimate stress changed but was not significant ($p=0.069$). Cross-sectional property changes were not significant from controls ($p=0.15$). Femur ash fraction for

hens in each dietary group showed a significant 2nd-degree polynomial trend ($p= 0.013$) when plotted against dietary calcium percentage (Fig. 3).

DISCUSSION

Egg production was not significantly different in the middle five of seven dietary calcium levels. These results supported our hypothesis that pheasant hens will produce the same approximate number of eggs, despite different levels of dietary calcium input. Only the 0.19% and 4.47% groups produced statistically fewer eggs than the other five levels. Dietary calcium of 0.19% most likely falls below a minimum threshold requirement for sustained egg production. Dale and DeWitt (1958) found that 0.5% dietary calcium was insufficient for laying hens and Chambers et al. (1966) found osteoporosis in hens at this level. Greeley (1962) found depressed egg production at the 0.37% level ($n=21$), but comparable production at the 0.63% ($n=55$) and 1.09% ($n=69$) levels. The 4.47% level probably exceeded the maximum threshold for calcium use in egg production (Hinkson et al. 1970, Reddy and Reddy 1985).

Cross-sectional properties of the cortical bone did not change. Avian bones are hollow and contain supporting struts, allowing minimum weight for flight without compromising skeletal strength. Natural selection may have pushed this adaptation to the limit, which our results support. Further thinning of the cortical bone may weaken long bones to the point where they would be easily fractured under wild conditions.

Ultimate load increased linearly with increased calcium ($p=0.005$). Femurs of pheasant hens on low calcium diets broke more easily under constant pressure. These results support our hypothesis. Hens in the middle five groups produced statistically the

same numbers of eggs. Those on lower calcium diets would only be able to do so if they utilized bone stores, which showed a positive correlation in the broken femurs as dietary calcium increased.

Femur ash fraction results reveal that the bone mineral content changed significantly during the experiment. Because pheasants could not draw on cortical bone for calcium without risking severe skeletal structure damage, they resorbed calcium in the medullary bone, which was reflected in the femur ash fraction. Reducing the mineral content of the femur allowed hens to mobilize calcium needed for egg production with minimal compromise to the skeletal structure. The consequences of drawing too much calcium in this fashion may affect skeletal integrity at only the lowest levels (below 1%).

We expected femurs of the 0.19% calcium level, which produced an average of only two eggs per hen, to more closely resemble normal femurs. Because these hens produced so few eggs, we expected that dietary calcium was not enough to stimulate enough estrogen for sustained ovulation and egg production. We predicted that hens were mobilizing medullary bone stores for simple maintenance needs over egg production and expected their femurs would be more difficult to break, like those of our control bones and the higher calcium levels femurs. However, this group produced the femurs most easily broken in our three-point bending tests. Our results suggest 0.19% calcium is insufficient for even maintenance physiological needs for hen pheasants in the breeding season, which is consistent with the literature (Dale and DeWitt 1958, Chambers et al. 1966, National Research Council 1994)

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We thank E. Sutherland and J. Bird for providing their pheasant care expertise, as well as the staff at Leland Mills for their help with the feed ration. We thank H. Cline for helping with pheasant care and egg measurements. We also thank R. Bogardus, K. Marvel, and J. Jorgensen, graduate students at Brigham Young University, for processing pheasant femurs. Thanks also to the lab of Bruce Webb for analyzing feed diets. We thank K. Simoni, at the Donahue lab at Michigan Technological University for analyzing the cross-sectional properties of our pheasant bones.

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TABLES

Table 1. Ingredients and nutrient contents of commercial and experimental diets

given to seven laying hen pheasant groups.

| <i>Ingredient</i> | <i>Diets g/100 g</i> | | | | | | |
|--------------------------|-----------------------------|------|------|------|------|------|------|
| | Control | 1 | 2 | 3 | 4 | 5 | 6 |
| Corn, yellow ground | 65.0 | 62.4 | 60.6 | 59.5 | 57.2 | 56.5 | 52.4 |
| Soybean meal (44%) | 27.5 | 27.5 | 27.5 | 27.5 | 27.5 | 27.5 | 27.5 |
| Wheat bran | 6.25 | 6.25 | 6.25 | 6.25 | 6.25 | 6.25 | 6.25 |
| Salt | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Vitamin premix* | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Dicalcium phosphate | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Calcite | 0.0 | 2.6 | 4.4 | 5.5 | 7.8 | 8.5 | 12.6 |
| Total | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| <i>Analysis</i> | | | | | | | |
| Protein | 19.2 | 19.0 | 18.8 | 18.7 | 18.5 | 18.4 | 18.0 |
| Methionine-Cystine | 0.61 | 0.60 | 0.59 | 0.59 | 0.58 | 0.58 | 0.56 |
| Lysine | 1.01 | 1.00 | 0.99 | 0.99 | 0.98 | 0.98 | 0.97 |
| Calcium | 0.19 | 1.07 | 1.68 | 2.05 | 2.84 | 3.08 | 4.47 |
| Phosphorus | 0.47 | 0.47 | 0.46 | 0.46 | 0.46 | 0.45 | 0.45 |

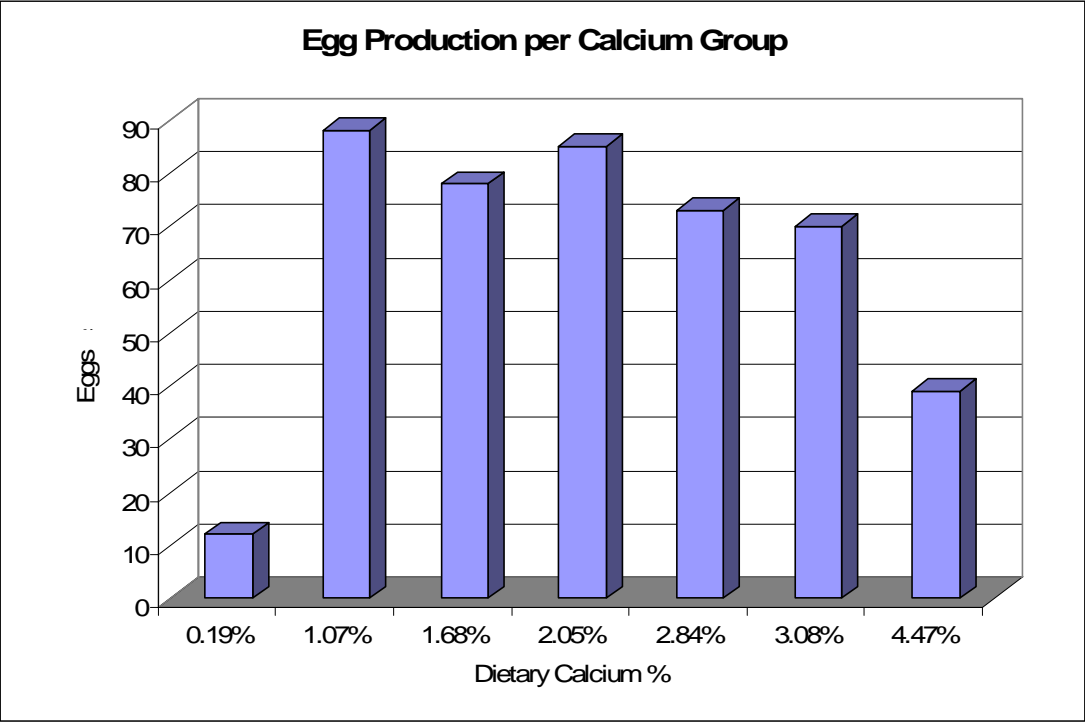
*Premix supplies per kg of diet the following amounts: calcium 0.34 g, magnesium 0.01 g, phosphorus 0.01 g, potassium 0.01 g, copper 4.4 mg, iodine 2.27 mg, iron 38.13 mg, manganese 66.0 mg, selenium 0.10 mg, zinc 60.03 mg, vitamin A 6666.0 IU, vitamin D 3305.5 IU, vitamin E 8.26 IU, niacin 22.01 mg, riboflavin 3.85 mg, pantothenic acid 5.4 mg, folic acid 0.23 mg, choline 274.92 mg, vitamin B12 1.0 mcg, pyroxidine 1.09 mg, menadione 0.55 mg, thiamine 1.45 mg.

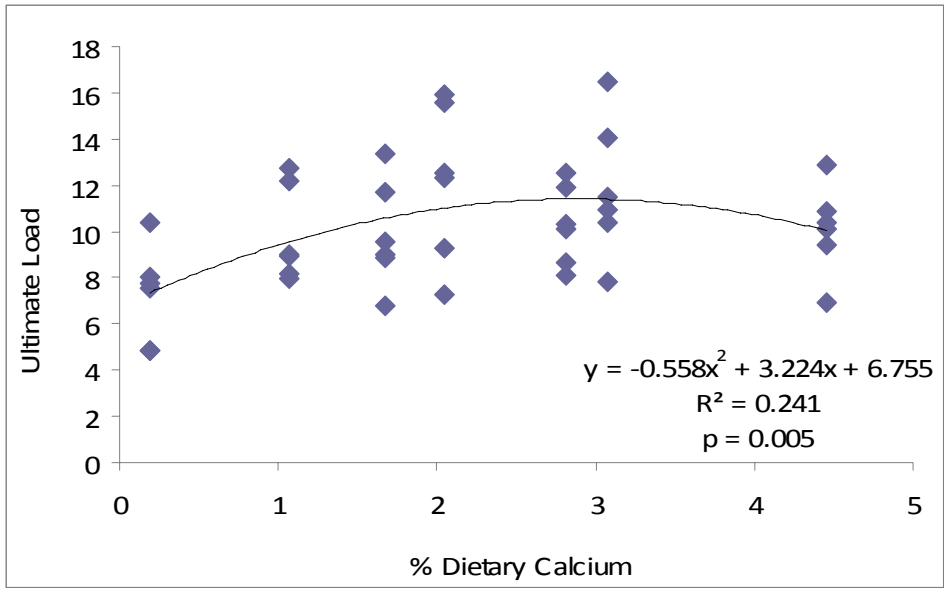
FIGURES

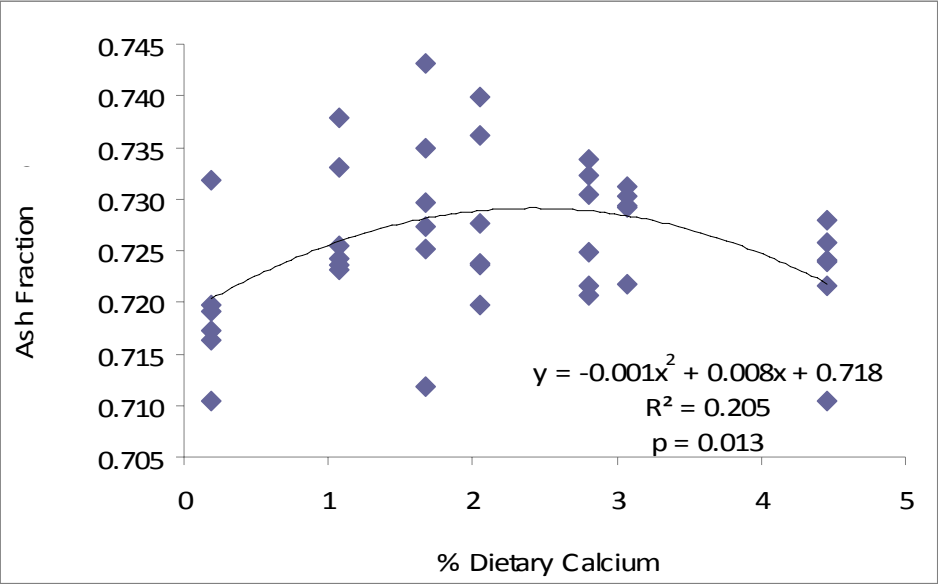
Figure 1. Eggs produced by pheasant hens given seven different calcium diets.

Figure 2. Ultimate load, normalized for body weight, for pheasant hens given seven different calcium diets.

Figure 3. Femur ash fraction (ash mass/dry mass) of hen pheasants given seven different calcium diets.







Physiological factors affecting eggshell coloration in Ring-necked Pheasants (*Phasianus colchicus*).

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Abstract

Ring-necked Pheasants (*Phasianus colchicus*) produce eggs that vary in color from blue-green to orange-yellow based on different amount of biliverdin (blue-green) and protoporphyrin (brown). We examined 437 eggs from 42 captive-bred hen pheasants given 7 different calcium diets to determine if egg color correlated with dietary calcium, egg mass, volume or shell thickness. We also compared pheasant egg color variation to a previous study. In all, 97.5% of pheasant eggs were brown, 2.5% were blue-green, suggesting that protoporphyrin is more abundant than biliverdin. Yellow pigment decreased with increasing calcium. Yellow pigment, orange pigment or darkness was not associated with egg characteristics measured. Orange pigment or darkness was not associated with dietary calcium. We concluded that biliverdin, found only in the yellow pigment, had a higher affinity for calcium.

Introduction

Avian eggshell pigments exhibit considerable color variation, from the bright blues of American Robins (*Turdus migratorius*) and many passerine species to the white eggs of cavity nesters and many domestic chicken varieties (Kennedy and Vevers 1976). The

color pigments contributing to bird eggshells fall into 2 classes of compounds, porphyrins and bilins (Hill and McGraw 2006). Protoporphyrin is the porphyrin responsible for brown and black colors of eggshells. The bilins responsible for most eggshell coloration include biliverdin and its zinc chelate, associated with blue and green pigments (Hill and McGraw 2006). Kennedy and Vevers (1976) surveyed 108 taxonomically diverse species and characterized which eggshell pigments their eggs contained, as well as any trace pigments present. The Ring-necked, or Common Pheasant (*Phasianus colchicus*), as labeled in their study, contained all 3 of these pigments. These pheasants lay eggs of an olive-brown color (Kennedy and Vevers 1976) and they nest on the ground in lowland or agricultural habitat (Giudice and Ratti 2001).

Labisky and Jackson (1966), in contrast to Kennedy and Vevers (1976), found considerable variation in the egg coloration of Ring-necked Pheasants. They examined the coloration of 630 eggs laid by 9 yearling hens in captivity, according to the color system established by Maertz and Paul (1950). They divided color pigments into 7 major color groups: red to orange, orange to yellow, yellow to green, green to blue-green, blue-green to blue, blue to red, and purple to red. Labisky and Jackson found that 98% ($n=619$) of the eggs laid by the yearling hens were in the orange to yellow color group. The remaining 2% consisted of 10 eggs in the yellow to green group and 1 egg in the green to blue-green group. Four of their 9 hens laid eggs in 2 different color groups.

Mixtures of differing amounts of the 3 major porphyrins could account for the color variation in Ring-necked Pheasant eggs that Labisky and Jackson (1966) found. Their results suggest that the brown color of protoporphyrin is usually more concentrated than biliverdin and biliverdin zinc chelate in this species, since hues of brown were only

in Maertz and Paul's (1950) orange-yellow color group. In the aberrant eggs, biliverdin and its zinc chelate were probably more concentrated. American Robin (*Turdus migratorius*) eggs, for example, are bright blue and contain only biliverdin (Kennedy and Vevers 1976). It is not known which color is more important nor if they are associated with physiological factors. Such knowledge may further understanding of timing and coloration mechanisms in the eggshell formation and pigmentation process.

Methods and Materials

We obtained 437 Ring-necked Pheasant eggs that were collected daily from 10 March to 9 May 2007 from 7 groups of 6 yearling hens. Each group was given diets differing in calcium percentage: 0.19%, 1.07%, 1.68%, 2.05%, 2.84%, 3.08% and 4.47%. Each egg was matched to the appropriate corresponding color in the Maertz and Paul (1950) system following Labisky and Jackson (1966).

Maertz and Paul's (1950) system matched color pigments to 3 quantifiable variables within each of their color groups. The first 2 variables follow a grid system on a single page called a plate. The horizontal axis at the top and bottom contained letters from A to L and the vertical axis contained numbers from 1 to 12. Yellow increased in slight increments across the page from the letter "A" as the horizontal axis approached the letter "L." Likewise, orange increased down the page from the number "1" as the vertical axis approached the number "12." The grid square "L1" represented the most yellow pigment and the grid square "A12" represented the most orange pigment in the color group. The third variable was the degree of darkness of each pigment in the color group. There were 8 plates per color group in the Maertz and Paul system with each

slightly darker than the previous plate. Plates 9 to 16 were in the orange to yellow color group. Plate 9 represented the brightest orange to yellow colors on its plate, with no gray color added to make any of the colors darker. Plate 10 contained the same colors and grid squares as Plate 9, but with a slight amount of gray added to each grid square. Each plate became increasingly darker with more gray added, up to Plate 16, the darkest of the color group.

Almost all (98%) of Labisky and Jackson's (1966) data set was in the orange to yellow color group. We limited our data to match the Labisky and Jackson sample for multivariate analysis. We matched our all eggs in the orange to yellow color group under uniform fluorescent lighting. Each egg contained a corresponding letter, number and plate associated with a single grid square. We compared the following variables to the degree of yellow pigment (Letter), orange pigment (Number) and darkness (Plate): dietary calcium percent, egg mass, volume and shell thickness.

Egg mass was determined on an electronic balance to the nearest 0.1 g. Egg volume was determined in a graduated cylinder to the nearest 0.5 ml by water displacement. Shell thickness was measured with electronic vernier calipers to the nearest 0.001 mm. The shell membranes were removed mechanically and the average of 2 measurements were taken at either end of the egg and at the midpoint, then averaged for the overall shell thickness measurement according to Greeley (1962). Letters were converted from alphabetic to appropriate numerals (A=1, B=2, C=3, etc.) for quantitative analysis. Plates in the orange to yellow color group were designated 9-16 and Numbers were already quantifiable for analysis in their present form (1-12). Multivariate statistical analyses were conducted in SAS Version 9.1 (SAS Institute Inc., Cary, NC). We also

surveyed the variation in our data set to compare with the results of Labisky and Jackson (1966).

Results

Of the 437 eggs from 42 yearling pheasants, we found 426 eggs (97.5%) in the orange to yellow color group 9 eggs (2.06%) in the yellow to green color group, 1 egg (0.23%) in the blue to green color group and 1 egg (0.23%) in the blue-green to blue color group (Fig. 1). All 7 calcium groups produced eggs in the orange to yellow color group. 5 of the 7 calcium groups produced at least 1 egg in the yellow to green color group; only the 0.19% and 3.07% groups did not. Only 1 calcium group (2.05%) produced an egg in the green to blue-green color group and was also the only group to produce an egg in the blue-green to blue color group.

Results of the multivariate analysis are shown in Table 1. Egg mass, egg volume and shell thickness were not significant at the 0.01 level for Letter, Number or Plate. For dietary calcium percent, only Letter (Yellow pigment) was found to be significant at the 0.01 level ($p < 0.0001$). As calcium percent increased, Letter decreased. At first glance, dietary calcium seemed to affect Number (orange pigment, $p = 0.041$) and Plate (darkness, $p = 0.039$), but the means of these trends did not show a clear increasing or decreasing trend as dietary calcium increased, as they did for Letter (yellow pigment, Table 2). The average Letter, Number and Plate values for each dietary calcium group are shown in Table 2.

Discussion

The variation we found among the color of yearling pheasant eggs seems to closely corroborate the findings of Labisky and Jackson (1966, Fig. 2). Their experiment yielded almost 200 more eggs than we had; however, we surveyed eggs from 42 pheasants instead of 9. We also found that our pheasants produced 98% of their eggs in the orange to yellow color group. We had 9 eggs in the yellow to green color group versus their 10. Both experiments yielded 1 egg from the green to blue-green group. Our experiment also yielded an egg in the blue-green to blue color group, which is not unexpected due to our larger sample size of yearlings and higher probability of variation.

Five of Labisky and Jackson's (1966) 9 hens (56%) laid only in the orange to yellow color group. Only 2 of our 7 groups of hens (29%) laid exclusively in the orange to yellow color group. Unfortunately, we were unable to sequester individual hens so it was uncertain which hen laid which eggs. Because of this, we did not know if hens within the other 5 groups laid eggs exclusively in the orange to yellow color group. No hen in Labisky and Jackson's experiment laid eggs in more than 2 color groups. This is probably true for our hens as well. Only the 2.05% calcium group laid eggs of more than 2 color groups and the hens in that group only laid 1 in the yellow to green color group, 1 in green to blue-green, and 1 in blue-green to blue out of 80 total eggs laid. There is a 2.8% chance that 1 of our 6 hens in that calcium group produced at least 2 of the 3 eggs was in the orange to yellow color group.

Multivariate analysis (Table 1) revealed that color or darkness were not correlated with egg mass, volume or shell thickness, which reflected our expectations. For eggshell thickness, this suggests that pigmentation is shallow and occurs only on the surface of the

eggshell, which corroborates literature findings (Lieberman 1878, Fischer and Kogl 1923, and Kennedy and Vevers 1973).

Calcium percent correlated with yellow pigment (Letter), but not orange (Number), or darkness (Plate). Yellow pigment decreased as calcium increased. It seems logical that eggshell pigments, when diluted with increased calcium, which is white in color, would make the egg appear lighter. However, we expected this trend to occur equally across both orange and yellow pigments.

Kennedy and Vevers (1976) described the color of Ring-necked Pheasant eggs as “olive-brown,” indicating at least a slight greenish tinge to the dominant brown color. They found that these eggshells contained protoporphyrin, biliverdin, and biliverdin zinc chelate. Smaller amounts of the latter 2 pigments were responsible for the green tinged eggshell color. Because the greenish color of biliverdin and its zinc chelate represented a blend of the primary colors blue and yellow, these pigments were in the Maertz and Paul (1950) system’s orange to yellow color spectrum as yellow pigments. Since our statistical results indicated that yellow alone was significant as calcium in the eggshells increased, we concluded that either biliverdin, biliverdin zinc chelate or both had a higher affinity for calcium than protoporphyrin.

Kennedy and Vevers (1973) stated that both porphyrins and bilins have a high affinity for calcium, but not which ones are more strongly associated with it. Further research should focus on finding which blue-green pigment, biliverdin, biliverdin zinc-chelate or both, is more strongly associated with calcium. Answers to these questions may help further illuminate unknown or untested details in the eggshell formation process, as well as the role and timing of pigmentation in this process. These answers

may also aid in further defining characteristics and roles of protoporphyrin and the bilins that give eggshells their various colors.

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Table 1. Multivariate results for each variable showing the relationship between Letter (yellow pigment), Number (orange pigment) and Plate (darkness), given as *p*-values.

Statistically significant results ($p < 0.01$) are bolded.

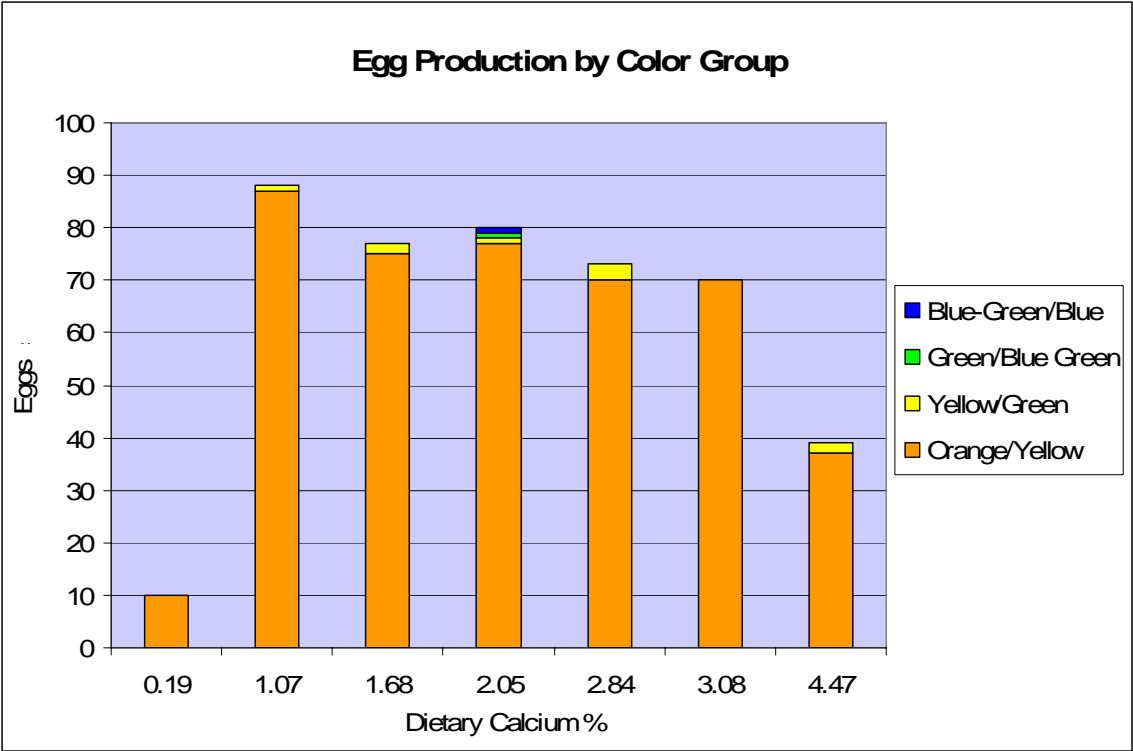
| | Yellow (Letter) | Orange (Number) | Darkness (Plate) |
|------------------------|------------------------|------------------------|-------------------------|
| Calcium % | <0.0001 | 0.041 | 0.039 |
| Egg Mass | 0.364 | 0.946 | 0.313 |
| Egg Volume | 0.466 | 0.825 | 0.031 |
| Shell Thickness | 0.266 | 0.831 | 0.552 |

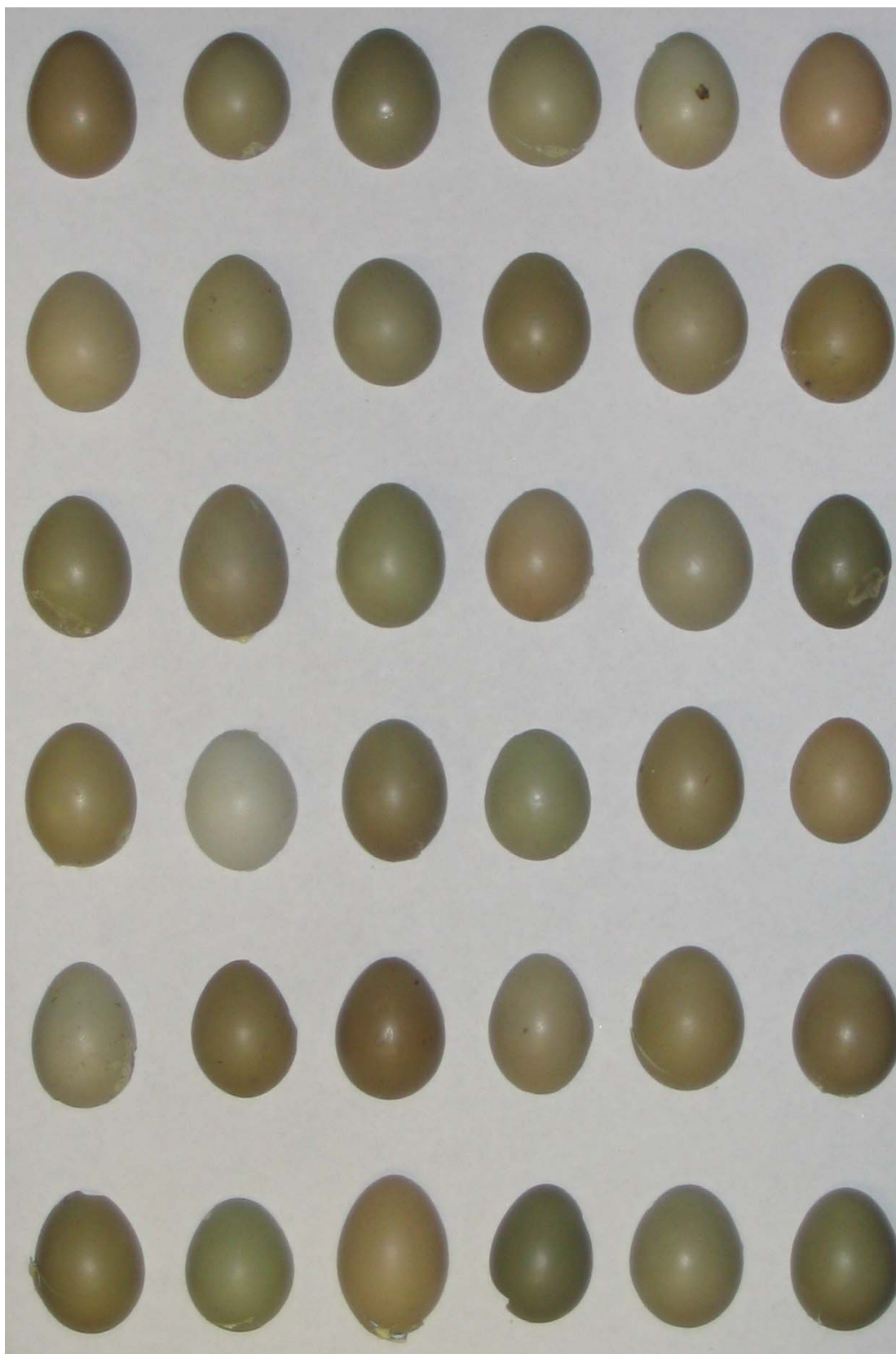
Table 2. Average Letter, Number and Plate values for each dietary calcium group.

| Calcium Group | Yellow (Letter) | Orange (Number) | Darkness (Plate) |
|----------------------|------------------------|------------------------|-------------------------|
| 0.19 | 5.20 | 5.80 | 12.60 |
| 1.07 | 5.09 | 4.24 | 12.77 |
| 1.68 | 4.66 | 4.20 | 12.84 |
| 2.05 | 4.15 | 3.74 | 13.06 |
| 2.84 | 4.40 | 4.22 | 12.90 |
| 3.08 | 4.34 | 4.26 | 12.59 |
| 4.47 | 3.26 | 4.44 | 12.64 |

Figure 1. Eggs produced by pheasant hens in 7 dietary calcium groups, divided into color groups according to Maertz and Paul (1950).

Figure 2. Several Ring-necked Pheasant (*Phasianus colchicus*) eggs laid by hens in our experiment, exhibiting high color variation.





RRH: SHORT COMMUNICATIONS

Does reducing the surface area of one wing of male Ring-necked Pheasants (*Phasianus colchicus*) induce unbalanced pectoral or humerus development after wing-whirring?

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ABSTRACT.--- Male Ring-necked Pheasants (*Phasianus colchicus*) perform wing-whirring frequently during the breeding season. We predicted that reducing one wing's surface area by half or more by clipping feathers, that constant wing-whirring for an extended period of time would exhibit reduced pectoralis muscles and humerus density between sides. We weighed pectoralis muscles and conducted three-point bending tests on humeri of seven pheasant males that performed wing-whirring over a 2-month period. We found no difference in pectoralis muscles mass, humeri breaking strength or ash fraction between reduced and unreduced wings. Wing-whirring may only put a negligible strain on male Ring-necked Pheasant pectoralis muscles and humeri, which have evolved to sustain the massive stresses and energy required for flight.

Ring-necked Pheasants (*Phasianus colchicus*) are dimorphic game birds that utilize a polygynous mating strategy (Giudice et al. 2001). The males are colorful and perform a variety of displays and vocalizations to compete for females during the mating season (Baskett 1947, Kozlova 1947, Taber 1949, Heinz and Gysel 1970, Cramp and Simmons 1980, Hill and Robinson 1988, Johnsgard 1999). Wing-whirring occurs when a male stands upright in a prominent location, spreads his wings, and flaps them vigorously in front of him for several seconds (Johnsgard 1999). During the peak of the mating season, males may perform wing-whirring every 10-15 min (Johnsgard 1999). Caged domestic chickens increased humerus bone density after 35 weeks of minimal exercise (Johnston et al. 2006). We predicted that if we reduced the surface area of one wing by half or more, constant wing-whirring over an extended period of time would cause reduced pectoralis muscles and humerus density between sides.

METHODS

We collected data and made observations during an experiment on Ring-necked Pheasants from 10 March to 9 May 2007. At the beginning of the experiment, we clipped the primaries of the left wing of seven males and housed them separately in seven pens with six females each. Most observations were in the late afternoon or early evening, but we also observed pheasants during the day for the 2-month period. Males began wing-whirring consistently during the last 2 weeks of March, when the females began laying and the males were constantly copulating with them.

We heard and observed males wing-whirring several times in 10 min among all seven pens. Because the pens were arranged in a line with no partitions, the males could

see each other and all but the end pens were directly adjacent to two other pens, with only chain-linked panels between them. Males were rotated once a week to ensure a uniform effect on fertility among the pens in the experiment. These conditions seemed optimal for maximum mating and dominance displays, although none of the males were in the same pen simultaneously. Males were often observed displaying and walking parallel (Johnsgard 1999) where neighboring pens joined. On several occasions, in late March and early April, we also observed a wild resident male displaying, walking parallel and pecking at the face of large males through the fence from outside of the pens.

At the termination of the experiment we euthanized all seven males. Pheasant 4 had molted early and the feathers on his left wing approximately matched those on his right wing. The other six pheasants' wings remained unchanged from the original trimming. We extracted the right and left pectoralis muscles from each male, freeze-dried them and measured their respective mass to the nearest 0.1g. We traced the right and left wing profiles of each pheasant on paper, blocked the sections into geometric shapes and calculated the surface area of each wing according to Corvidae et al. (2006). We compared the mass of left and right pectoralis muscles of each bird with the surface area of their left and right wings in a Paired Two-sample *t*-test in Excel (Microsoft Corporation, Redmond, WA). We assumed that during wing-whirring, male pheasants flapped both wings equally, despite the reduced surface area of the left wing. Our observations seemed to verify this because the males did not look unbalanced or asymmetrical during wing-whirring.

We measured breaking strength of humeri of males 2, 3, 4, 5 and 7 in a three-point bending test with an Instron machine (3300 series, Canton, MA). Bones were stored

frozen with soft tissue intact. Soft tissue was removed immediately before breaking. Adjustable fulcra were positioned approximately 10 times the middiaphyseal diameter of the bone. Preload was set to 0.33 N. Loading rate was set at 10 mm/min as suggested by Lopez and Markel (2000). Bones were paired and broken in randomized order. Humeri were broken with the anterior surface in compression. Tests ended when maximum load dropped by 40%. Fulcra were wiped clean with an alcohol wipe before each specimen was broken. Bones were measured using a digital caliper and marked appropriately. Prior to testing, each bone shaft was wiped with a dry paper towel to remove any excess oil and water. Data were collected every 50 msec. Humeri were then placed in a muffle furnace for 12 hr and ash fraction values were determined.

RESULTS

Table 1 shows the values for pheasant pectoralis mass, humerus breaking strength, and surface area. We found no correlation between reduced wing surface area and matching pectoralis muscles ($p=0.486$), humerus breaking strength, ($p=0.486$), or ash fraction ($p=0.203$). Figure 1 shows results of the three-point bending test for Pheasant 4.

DISCUSSION

Several factors could account for the above results. Although each male pheasant may have wing-whirred several dozen times a day, one and a half months may not be enough time to make a difference in the mass of the pectoralis muscles or in humerus density. Johnston et al. (2006) found increased humerus density in chickens after 35 weeks, far longer than our experiment. Wing-whirring may only put a light or moderate

strain on the pectoralis muscle and humeri, which have evolved to sustain the massive stresses and energy required for flight. Domestic chickens have also undergone significantly more selection than Ring-necked Pheasants.

Clipping the primaries alone may not be enough to reduce pectoralis mass or humerus density on one side, but removing parts of the wing may result in significant reductions. Future experiments may explore this idea. Our results were also statistically limited by small sample size. Future experiments in more controlled environments with larger sample sizes would reveal more accurate results. Based on our data, reducing the surface area on one wing of male pheasants does not induce unbalanced pectoral or humerus development after wing-whirring.

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TABLES

Table 1. Pectoralis mass, humerus maximum load, and surface area measurements for the right (R) and left (L) wings of 7 male Ring-necked Pheasants. NV= no value. Pheasant 4 regrew its left wing primaries by the end of the experiment.

| Pheasant | Mass Pectoralis (g) | | Maximum Load (Mpa) | | Surface Area (cm) | |
|----------|---------------------|-------|--------------------|--------|-------------------|--------|
| | R | L | R | L | R | L |
| 1 | 16.20 | 16.30 | NV | NV | 516.12 | 325.32 |
| 2 | 15.40 | 16.00 | 195.52 | 189.52 | 537.17 | 333.66 |
| 3 | 16.80 | 16.60 | 124.10 | 140.81 | 553.84 | 235.32 |
| 4 | 11.90 | 10.80 | 166.94 | 170.92 | 564.02 | 506.86 |
| 5 | 15.40 | 14.70 | 181.72 | 174.80 | 573.01 | 357.04 |
| 6 | 13.20 | 15.20 | NV | NV | 524.34 | 294.66 |
| 7 | 14.00 | 13.20 | 171.77 | 163.14 | 551.18 | 259.33 |

FIGURES

Figure 1. Three-point bending curves for the left and right humeri of Pheasant 4, showing almost identical breaking trends and maximum loads. The thicker line represents the right humerus, the thinner line is the left humerus. Triangles represent maximum loads.

