Reproductive Soundness and Egg Quality in Chickens Selected for Low and High Antibody Response

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ABSTRACT

For 36 generations, White Leghorn chickens were selected for high (HAS) or low (LAS) antibody response to sheep red blood cells. The focus of this thesis was to investigate correlated responses in reproductive soundness and egg quality resulting from that selection. Forty-five hens and 25 roosters from each antibody line were used. In hens, commencement and intensity of lay, and egg quality, were analyzed; in both sexes, length of fertility was considered. Hens and roosters were mated to an intercross line to avoid confounding selection with sex effects. The LAS line was more reproductively sound, commencing lay at a younger age (11.67 ± 3.53 d; $P < 0.001$), lighter body weight (-169.46 ± 40.20 g; $P < 0.001$) and with greater intensity (2.68 ± 0.25%; $P = 0.001$) than the HAS line. Additionally, the LAS line had a greater length of fertility (hens: 2.43 ± 0.55 d; $P < 0.001$; roosters: 3.11 ± 0.71 d; $P < 0.001$). In contrast to their poorer reproductive soundness, the HAS line had superior egg quality compared to the LAS line. Egg shape index (4.12 ± 0.55; $P < 0.001$) and albumen height, measured in both mm (0.27 ± 0.12 mm; $P < 0.001$) and Haugh units (1.89 ± 0.91; $P = 0.04$), were superior in HAS hens. Selection for increased antibody response appeared to compromise reproductive soundness, perhaps due to limitations in available resources. However, the selection did not compromise egg quality.

Key words: chickens, egg quality, reproductive soundness, antibody response

DEDICATION

To my grandparents, Joan and Russ Mosher (a.k.a. Mimi and Bampa).

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AH	albumen height
AI	artificial insemination
BW	body weight
C	control
cc	cubic centimeter
cm	centimeter
CP	crude protein
d	day
EL	egg length
ELISA	enzyme-linked immunosorbent antibody assay
EST	eggshell thickness
ESW	eggshell width
EW	egg width
g	gram
h	hour
HAR	high antibody relaxed
HAS	high antibody select
HU	Haugh unit
HWS	high weight select
IC	intercross
kcal	kilocalorie
kg	kilogram
L	low
LAR	low antibody relaxed
LAS	low antibody select
LWS	low weight select
µ	micro
ME	metabolizable enerty
MG	*Mycoplasma gallisepticum*
mL	milliliter
mm	millimeter
mo	month
pH	percentage Hydrogen
ppm	parts per million
SG	specific gravity
SI	egg shape index
SP	shell percentage
SRBC	sheep red blood cell
wk	week
WT	egg weight
YC	yolk color

Chapter 1: General Introduction and Review of Literature

Heather N. Albrecht

Introduction

Reproductive soundness and egg quality are important economic drivers of commercial poultry operations. Reproductively sound chickens are typically more efficient, helping to maximize production and minimize cost. Superior egg quality is also important to ensure a consistent and high quality food for processors and consumers.

Consumers are constantly increasing demand for changes in available products. With the consumer as a driving force of the poultry industry, when possible, producers supply products to meet those demands. Many of the desired changes can be made by implementing a genetic selection program. However, selection to improve certain production traits may adversely affect others. Genetic improvement does not come without cost.

Chickens, like other animals, allocate nutritional resources to different physiological activities including maintenance, growth, reproduction, immunity (Siegel and Honaker, 2009) and social interactions. Genetic selection can cause changes in the allocations of these resources, favoring some physiological traits or functions while disadvantaging others (Gross et al., 2002). A decrease in resources available may produce unfavorable results, such as decreased reproductive soundness or reduced quality and efficiency of production.

It is important to understand how selection on one or a few traits influences other aspects of the animal. The objective of this study is to examine the consequences of long-term selection in White Leghorn chickens for high (**HAS**) or low (**LAS**) antibody response to sheep red blood cells (**SRBC**) on reproductive soundness, egg quality and antibody response to *Mycoplasma gallisepticum* (**MG**). As an outcome, our understanding of trade-offs resulting

from selection for antibody response on other aspects of production efficiency in poultry operations will be improved.

Divergent Selection of White Leghorn Lines to Sheep Red Blood Cells

White Leghorn chickens divergently selected for high and low antibody response to SRBC were used to examine production intensity, fertility, egg quality traits and resistance to MG. The comparison of the 2 White Leghorn lines was facilitated by a control line of intercrossed White Plymouth Rock chickens to independently evaluate fertility for both roosters and hens. The White Plymouth Rock chickens used were an intercross (**IC**) line of 2 lines divergently selected for high and low 8 wk BW. The following section of this review discusses the development of the lines, as well as the procedure for measuring the antibody response to SRBC.

Development of High and Low Antibody Lines. The Cornell Randombred population of White Leghorn chickens was the foundation stock of the HAS and LAS lines (King et al., 1959). These antibody lines were established by selecting chickens with the most extreme response to SRBC from the foundation stock, and using those chickens to parent the S_1 generation (Siegel and Gross, 1980; Siegel et al., 1982). Subsequent generations were formed using truncation selection in regards to the phenotype of individuals within each line; each generation, 7 roosters and 28 hens were used to generation S_5. Thereafter, the lines were reproduced by 8 and 32 roosters and hens, respectively. In each case, pedigree mating involved 1 rooster to 4 hens.

To determine phenotype, antibody response was measured using the procedure of Wegmann and Smithies (1966). Chickens between 6 and 8 wk of age (Martin et al., 1990) were injected intravenously with 0.1 mL of a 0.25% suspension of SRBC. Five d later, a tube dilution

procedure was used to determine antibody response from collected plasma; the titers were expressed as the $\log_2$ of the reciprocal of the last dilution with a positive response, or agglutination (Siegel et al., 1980). In the S_3 generation, the microtiter method became available. Since this method was both quicker and less expensive, it was adopted thereafter to quantify antibody response. The correlation between titers obtained from the 2 procedures was tested both within lines and sexes. The average correlation coefficient was 0.82, and the results from both procedures deemed sufficiently similar (Siegel et al., 1982).

Each generation, chicks from both antibody lines were hatched on the same day in March, vaccinated for Marek's disease, and placed in group pens on littered floors with hot-air brooding. Food and water were given *ad libitum* and males and females were intermingled (Siegel et al., 1980; Martin et al., 1990). At 18 wk, chickens were moved to individual cages (Martin et al., 1990).

When the lines were initially established, the HAS and LAS lines separated quickly with regards to their response to SRBC, and have continued to diverge over many generations. After generation 14, there was very little overlap of the range of the titers expressed between the HAS and LAS chickens (Martin et al., 1990). As of generation 30, there was more than a six-fold difference between the average responses of the HAS and LAS lines to the SRBC (Kuehn et al., 2006). The divergence over the 30 generations can be seen in Figure 1.1. Also in the figure are 2 relaxed sublines that were established in generation 24. The relaxed lines were established by randomly selecting 10 roosters and 20 hens from each of the antibody lines (Kuehn et al., 2006). The relaxed lines showed that random mating within a single line causes both antibody lines to produce responses to SRBC that regress back towards the original response of the lines. This suggests that natural selection favors an intermediate response to SRBC (Kuehn et al., 2006).

Notable about the pattern shown in Figure 1.1 are the "waves of response". These waves are from an intense increase in response, followed by a cessation, then a further increase in response. These patterns are frequent in long-term selection experiments, as a consequence of sensitivity to microenvironmental factors and spontaneous mutations (Dunnington and Siegel, 1996; Kuehn et al., 2006). The lines typically recover quickly from such mutations, resuming the generalized direction in increase or decrease in antibody production. Over the course of a long-term selection experiment, mutations can arise sporadically more than once, resulting in such wave-like patterns. In addition, such "waves" may result from changes in gene networks and epistasis.

Development of Control Line. The IC line, which was used in this experiment as a control line to help differentiate between hen and rooster aspects of fertility, was created in a similar fashion to the antibody lines. White Plymouth Rock chickens, developed at the Virginia Agricultural Experiment Station, formed the foundation stock from 7 inbred lines. From this group of chickens, those with the heaviest BW formed the parents for the high weight selection (**HWS**) line and those with the lowest BW formed the parents for the low weight selection (**LWS**) line. In generation 41, reciprocal crosses of these lines were made to produce an F_1 generation which, in turn, produced an F_2. Subsequent generations were produced at random with the restriction that mating of full sibs was avoided. The IC chickens used in this experiment were progeny from generation 12.

In the Netherlands, egg quality and hatchability have also been examined in lines divergently selected for their response to SRBC (van den Brand et al., 2004). The chickens used in that study were divergently selected for over 22 generations. Antibody response was measured in chickens that were 37 d old, 5 d after the challenge of an intramuscular injection of

SRBC (Parmentier et al., 1996; van den Brand et al., 2004). The originating population was of ISA Warren medium heavy layers. The 22 generations of selection produced 3 lines identified as high (**H**), control (**C**) and low (**L**). The objective of this experiment was to determine if lines that differed in their response to SRBC had differences in egg quality traits, which might lead to differences in hatchability (van den Brand et al., 2004).

Resource Allocations

Chickens, like all animals, partition resources for physiological functions. Poultry allocate nutritional resources for maintenance, growth, reproduction, immunity (Siegel and Honaker, 2009) and social interactions. When selection pressure is applied, the resources needed to support the changed performance for traits under selection must be reallocated from other physiological functions (Gross et al., 2002). This study examined the impact on reproductive soundness and egg quality when chickens were selected for antibody response to SRBC.

Egg Traits and Their Relative Importance

Many traits of the chicken egg can be measured or quantified to help determine its internal and external quality. Internal quality refers to the quality of the albumen and yolk including measures of weight, pH, thickness and color. External quality refers to the shape of the egg and measures of the eggshell. Egg traits are influenced by a variety of factors including genetics, hen age and BW, diet and length of holding period (Silversides, 1994; Monira et al., 2003; Silversides and Budgell, 2004; van den Brand et al., 2004). The traits described in this section include: egg shape index (**SI**), egg weight (**WT**), albumen height (**AH**), Haugh units (**HU**), yolk color (**YC**) and eggshell weight (**ESW**) and thickness (**EST**). Descriptions of the

traits examined were drawn from numerous studies. A summary of the details of each experiment (e.g. breeds used, ages of animals, traits examined) is available in Table 1.1.

Egg Shape Index. Shape index is a measurement of the overall shape of an egg. The 3 shapes most prevalent in production are classified as sharp (SI of < 72), normal (SI of 72-76), and round (SI >76). Egg shape is important in commercial systems, as shapes outside the normal range do not fit well into pre-made packaging. Also, sharp eggs are not as resistant to the shipping and handling processes, as are their normal counterparts (Altuntas and Sekeroglu, 2007). To calculate shape index, the EW and EL of the egg are measured in cm using calipers. The EW is then divided by the EL and that ratio multiplied by 100 (van den Brand et al., 2004).

Van den Brand et al. (2004) evaluated SI in the eggs from layers of ages ranging from 25 to 59 wk at 4-wk intervals. Approximately 20 to 30 eggs were collected for each line (H, C and L), from each sire family, at each age interval (25, 29, 33, 37, 41, 45, 49, 55 and 59 weeks); a total of 776 eggs was assessed (273 H, 276 C, and 236 L). Measurements were taken on the egg within 3 h of being laid. From the beginning to the end of the experiment, SI decreased steadily from 77.02 to 72.85 ± 0.29 ($P < 0.05$). When examined by line, all 3 lines showed differences in their average SI (L: 73.62; H: 74.54; C: 75.77 ± 0.17, for each mean) ($P < 0.05$). Each line averaged a SI classified as normal, though the C line fell near the threshold of round eggs.

Anderson et al. (2004) performed an experiment to determine if genetic selection influenced egg shape, among other eggshell characteristics in White Leghorn chickens. Three of the control strains (**CS5, CS7** and **CS10**) came from base populations from Agriculture Canada in 1950, 1959 and 1972, respectively. The fourth population was the current commercial "Nick Chick" 1993 commercial strain (**CCS**). This strain shared genetic lineage with the 3 control

strains. At 18 wk, all hens were housed in cages with 6 hens/cage in a tri-deck system. All lines were represented in all rows and levels. Egg collection and measurements were taken every 4 wk, starting at 28 wk and continuing through the second production cycle, ending at 86 wk. The continuous experiment covered 2 production cycles with a molt occurring between the cycles at 62 wk. The authors did not provide monthly measurements, but an overall long-term average. The average SI measurement in the group with a base population from 1950 was 71.54 and classified as sharp. Looking at the strains derived from base populations from 1959, 1972 and 1993, the average SI values increased in the more recent strains (1959: 72.48; 1970: 73.59; 1993: 74.76) ($P < 0.05$) indicating rounder, more normal, eggs. This increase in SI score indicates possible genetic selection for larger, more round eggs. While selection criterion was not specifically stated, this type of selection, over time, would to help to improve resistance to breakage in the shipping and handling of these table eggs.

An experiment conducted by Popova-Ralcheva et al. (2009), examined the effects of age and genotype on certain egg quality characteristics. The following lines and line combinations of chickens were used to evaluate eggs from hens at 32 and 50 wk: Red Broiler x White Plymouth rock mini, "C" line x White Plymouth Rock mini, Labelle (synthetic), Red Broiler x Labelle, "D" line x White Plymouth Rock, and Labelle x "C" line. Among many other traits, SI was measured and was shown to increase with the age of the hen. Shape index ranged from 75.88 ± 0.7 to 78.45 ± 0.7 in the eggs from the hens at 32 wk and from 73.46 ± 0.84 to 76.29 ± 0.52 in eggs from hens at 50 wk. In all 6 line combinations, there was a numerical decrease in SI from 32 to 50 wk, suggesting that as hens aged, eggs moved from rounder to more normal egg shapes.

Since SI is important for commercial industry, when considering pre-made packaging, normal shaped eggs are ideal for fitting into containers. Normal shaped eggs also provide more strength to the eggshell, compared to sharp eggs, making them more resistant to breakage during shipping and handling (Altuntas and Sekeroglu, 2007). Additionally, uniformity in egg shape is important, as the market for further processed eggs continues to grow. The efficiency of this market is based on the use of automatic breakers, and conformity in egg shape to the characteristics of this machinery is essential. Based on the literature, we hypothesized that the values of SI measurements in our study would move, over time, to optimize at a more normal egg shape. Because the eggs from these lines are not marketed, there is no direct selection for egg shape. However, natural selection may cause the eggs to move in the direction of more normal shape, as these have stronger eggshells than their more extreme counterparts. The findings of van den Brand et al. (2004), lead us to anticipate a decrease in SI, from sharp to normal, as hens age.

Egg Weight. Egg weight is a very simple measurement to collect and therefore is frequently analyzed. Egg weight is measured simply by placing an unbroken egg on a scale and recording the value. Genetics and environment greatly influence WT. Birds of heavier BW lay smaller eggs relative to their body size, while birds of lighter BW lay larger eggs relative to their body size (Hafez et al., 1955). Almost all egg quality traits decline as hens age, with the exception of WT, which increases (Ledur et al., 2002; Altunas and Sekeroglu, 2007).

According to Zeidler (2002), weights of eggs are divided into 6 size categories. Minimum WT requirements in the United States for these categories are: jumbo (68.6 g), extra large (61.5 g), large (54.4 g), medium (47.3 g), small (40.3 g), and peewee (no minimum requirement). Van den Brand et al. (2004) examined WT and found that it increased with the

age of the hen. The average WT of eggs from hens from their 3 lines at 25 wk was 49.21 ± 0.43 g. The WT steadily increased as hens aged, resulting in an average WT of 61.01 ± 0.43 g ($P < 0.05$) at 59 wk. Egg weight also differed between the antibody lines with the L line having heavier eggs than the H line (55.55 and 54.23 ± 0.25 g, respectively). The difference between the WT in the antibody lines may be indicative of a difference in resource allocation. Hens selected for a greater antibody response must invest greater nutritional resources to produce said response, thus limiting resources available to use in egg production, possibly resulting in smaller eggs compared to those from hens selected for low antibody response.

Tharrington et al. (1999) assessed the quality and composition of eggs as influenced by genetic selection. They compared the same 4 strains of hens as used by Anderson et al. (2004). A variety of egg measurements were taken once per mo over a period of 60 wk, with the initial measurements taken at 28 wk. Eggs were collected within 24 h of production, tested for specific gravity (**SG**) and dried and stored overnight a 5° C. The next day, a 10 egg sample from each of the 4 strains was weighed and broken for further analysis. Though their observed values were not reported, WT increased progressively within each of the 4 strains ($P < 0.05$). At 63 wk, the hens underwent a molt, which caused the increase in WT to level off. There was a difference between 4 strains for average WT (CS5: 58.57; CS7: 59.81; CS10: 62.91 and CCS: 63.88 (pooled SEM ± 0.18 g)) ($P < 0.05$). The increase in WT in the more recently developed strains shows that larger WT was desired and subsequently selected for.

Silversides et al. (1994) compared egg traits among lines of albino and non-albino chickens, using 2 commercial lines as controls. The objective of the study was to determine the effect of the sex-linked gene for imperfect albinism ($s^{al\text{-}s}$) on egg production. The hens in this study were produced using specific crosses. Roosters, which were heterozygous for the albino

gene, were crossed with hens from 2 lines which had been selected for increased egg production for 8 generations. These crosses resulted in albino and non-albino hens from each of the 2 lines. In addition to these 4 groups of hens, 2 commercial lines of hens were used.

At 17 wk of age, hens from all 6 lines were randomly caged in groups of 3, and blocked so that each hen could contribute only 1 egg per sampling period. Eggs were collected at 30, 45, 60, and 75 wk of age, and stored over night at 4° C. There was a steady increase in average WT with age of hen among all lines. The overall WT average at 30 wk was 52.56 ± 0.25 g, which increased over the 4 measurements to 60.13 ± 0.25 g at 75 wk ($P < 0.05$). In support of Tharrington et al. (1999), average WT increased numerically across the 4 measurements, though the increase was not significantly different between the last 2 measurements, signaling a leveling off. When comparing lines, the control lines had heavier eggs on average than the other 4 lines, with average WT of the 2 control lines being 60.60 ± 0.30 g and 64.22 ± 0.30 g ($P < 0.05$), respectively.

The above studies all suggest an increase in WT with increased age. While advancing age causes WT to increase, the rate of increase is reduced with time. We presumed that WT in our experiment would be the lowest early in production and increase thereafter.

Albumen Height. Albumen refers to the "white" of an egg and consists of a thick and thin portion. The thick albumen is the portion immediately surrounding the egg yolk, whereas the thin albumen comprises the rest of the white portion. The height of the albumen indicates the freshness of the egg and can be measured using a tripod micrometer. Once the egg is broken onto a flat surface, a tripod micrometer is placed over the thick albumen. The center pin is lowered until it "kisses" the albumen and the height, typically in mm, can be observed. The

thicker the albumen, the better the quality of the egg, with heights of 8 to 10 mm being considered superior interior quality (Zeidler, 2002).

While AH can be measured directly, an additional measure of AH, HU, which accounts for WT, can be calculated (Haugh, 1937; Williams, 1992). The calculation for HU is as follows:

$$HU = 100 \log(AH - 1.7WT)0.37 + 7.57$$

where AH is the height of the albumen in mm and WT is the weight of the egg in g. Since the relationship between WT and AH is not constant across lines of birds, the HU is not appropriate for comparing eggs across lines (Silversides, 1994).

The age of hen and laid egg are important influences on AH. The length of time a hen has been in continuous production, or has continuously produced eggs without going through a molt, will impact the height of the albumen. Furthermore, AH will decrease as a hen ages (Doyon et al., 1986; Williams, 1992; Silversides, 1994; Ledur et al., 2002; van den Brand et al., 2004). As a laid egg ages, the AH also will decrease (Silversides, 1994; Silversides and Budgell, 2004).

Besides SI and WT, van den Brand et al. (2004) evaluated AH. In the youngest hens, the average AH was 7.27 ± 0.18 mm, while the average AH in the oldest hens decreased by 1.78 ± 0.18 mm ($P < 0.05$). When comparing overall AH averages across lines, the L and C lines had similar AH of 6.33 ± 0.10 mm and 6.00 ± 0.10 mm, respectively ($P > 0.05$). The H line had a lower average height (5.38 ± 0.10 mm) compared to the L and C lines ($P < 0.05$). Over time, the C line had the fastest decrease, suggesting that those hens were more sensitive to the ageing process. Also notable, the AH of the line H was lower than that of line L for the length of the

study. This reflects differences in resource allocation between hens selected for their antibody response.

Silversides et al. (1994) also measured AH at 30, 45, 60, and 75 wk in their albino, non-albino and commercial control lines of chickens. Eggs were broken onto a flat surface and the height of the thick albumen measured. The average AH, across strains, steadily decreased as the age of the hens increased ($P < 0.05$), with average values at 30 and 75 wk of 6.70 ± 0.06 mm and 5.50 ± 0.06 mm, respectively. Over the course of the experiment, average AH ranged from 5.50 ± 0.1 mm in a non-albino strain to 6.70 ± 0.1 mm in a commercial strain. The 2 commercial strains had larger AH measurements than all 4 selection lines of albino and non-albino chickens ($P < 0.05$).

Silversides and Budgell (2004) obtained eggs from hens from ISA Brown and Babcock B300 commercial lines, and from hens of a Brown Leghorn line that had had no selection since 1965. Eggs were collected at 32, 50, and 68 wk of age to represent early, middle and late stages of production, respectively. The focus of the study was to determine the significance of the genetics, hen age, and storage time on quality aspects including AH, pH, and whipping volume of the albumen. Eggs were measured within 2 h of being laid and after being stored for 5 and 10 d at 21°C. Albumen height in eggs of hens at 32 wk of age was 6.47 ± 0.06 mm. In hens at 50 and 68 wk of age, AH had a decreased of 0.71 and 1.71 ± 0.06 mm, respectively ($P < 0.05$). Measurements also decreased with increased storage time. Average AH for eggs measured within 2 h of production across ages and lines was 8.45 ± 0.06 mm. A decrease of 3.49 and 4.35 ± 0.06 mm ($P < 0.05$) in AH, respectively, was seen in eggs stored for 5 d and 10 d. This decrease in AH with increased storage time supports the premise that AH is an indicator of the freshness of the egg, with larger values indicating fresher eggs.

Among other egg characteristics, Monira et al. (2003) also assessed AH in chickens (Barred Plymouth Rock, White Leghorn, Rhode Island Red, and White Rock) ranging in age from 220-260 d. The focus of the study was to examine internal and external traits of the 4 breeds under storage times of 1, 7, 14 and 21 d. Twenty eggs were collected from each of the 4 lines and stored at 27.40 ± 1.25° C and 80.50 ± 1.90% relative humidity. After 1 d, 5 eggs from each breed were evaluated for various characteristics. The same evaluation was repeated after 7, 14 and 21 d of storage. As expected, there was an effect of the holding period. The average AH decreased in the eggs of all 4 breeds as the holding period increased ($P < 0.001$), indicating a reduction in freshness.

Previous research has shown that AH of eggs decreases as hens age and with increased length of storage. We suspected AH measurements of the eggs in this study would be the thickest during early production and decrease thereafter as the age of the hens increase. In the current study, eggs were analyzed within 24 h of production, minimizing any effects of storage time.

Eggshell Weight. Eggshell weight is the weight of the shell portion of an egg alone, although the procedures for obtaining that weight vary. First, the eggshells can either be rinsed out by hand or simply set upside down and allowed to drain. Next, the membranes inside of the eggshell can either be included in the weight or removed prior to weighing; if removed, such is typically done during rinsing. Lastly, eggshells are usually dry when weighed and can be dried either by air, a fume hood (Anderson et al., 2004) or in an oven at 100° C (Silversides, 1994). Egg shell weights have been shown to both increase and decrease as the hen ages, following no specific pattern (Silversides, 1994; Silversides and Budgell, 2004 Popova-Ralcheva et al., 2009)

In the study by Anderson et al. (2004), where 4 lines of White Leghorn chickens were compared, ESW was also measured. Like WT, as discussed previously, ESW was heavier in the lines more recently established. The oldest line had the lightest average ESW of 5.28 g. The second oldest line had a slightly heavier average ESW, though not significantly different from the oldest line. Each of the 2 younger lines had significant increases in average ESW (CS10: 0.35; CCS: 0.56 g) when compared to the oldest line ($P < 0.05$). These increases in ESW are due, in part, to the selection for heavier WT over time.

In the experiment comparing albino and non-albino lines of layers to control commercial layers (Silversides, 1994), ESW was also measured. At 30 wk, the average ESW was 5.44 g, with an increase of 0.15 g at 45 wk ($P < 0.05$). Eggshell weight then decreased at both 60 and 75 wk with average weights of 5.50 g and 5.35 g, respectively ($P < 0.05$). When the 6 strains were compared, the 2 commercial strains had significantly heavier eggshells than the selection lines with ESW measures of 5.88 ± 0.04 and 6.13 ± 0.04 g ($P < 0.05$). The increased weight of the eggshells in the commercial strains was consistent with the increased WT of the same lines.

Popova-Ralcheva et al. (2009) also evaluated ESW. Eggshell weights varied from the measurements taken at 32 wk to those taken at 50 wk. Similar to Silversides (1994), ESW increased numerically in 4 strains as hens aged, with increases of 0.32 to 1.18 ± 0.20 g. 2 groups, however, had decreases in ESW (0.04 and 0.23 ± 0.18 g) with an increase in hen age.

The results from studies examining ESW were inconsistent. Anderson et al. (2004) found that hens from more recently established lines laid eggs with heavier shells than the hens from older lines. Silversides (1994) found that ESW varied with the age of the hen. At first it increased as the hen aged but then decreased after 60 wk. This experiment also showed that

commercial-strain hens laid eggs with shells that were heavier than those of hens selected for or against albinism. Popova-Ralcheva et al. (2009) also observed ESW vary across lines and ages, but with no specific pattern. Some of the variation in ESW may be due to the varying egg weight (Silversides et al., 1994). Defining firm expectations as to the changes expected in ESW in the antibody lines in the current study was difficult.

Eggshell Thickness. According to Zeidler (2002), eggshell strength is highly dependent on EST. The EST measurement typically has little variation across similar breeds (Potts et al., 1974; Anderson et al., 2004). Literature suggests that EST can either decrease (Anderson et al., 2004) or remain constant as hens age (van den Brand et al., 2004). The thickness of an eggshell can be compromised in a variety of ways, including temperature over 32°C, hen age, and dietary calcium levels below 3% (Zeidler, 2002). An EST of at least 0.33 mm has been estimated to be necessary for the egg to have at least a 50% chance of withstanding normal handling conditions without breaking (Stadelman, 1995). Thickness measurements are typically taken along the midline of the egg and done using a micrometer. Eggshell thickness can only be evaluated after an egg has been broken.

In the study conducted by van den Brand et al. (2004), EST did not change as the age of the hen increased ($P > 0.05$); however, there were differences between the 3 selection lines. The H line had the thickest eggshells (0.344 ± 0.003 mm) and the L line had the thinnest eggshells (0.295 ± 0.003 mm), with the C line intermediary ($P < 0.05$). This distribution corresponds with that of eggshell percentage (**SP**). Eggshell percentage, defined as the weight of the eggshell as a percentage of the total WT, was the greatest in the H line (12.87 ± 0.11 %) , indicating thicker shells, as compared to the L line, which had the lowest SP (12.36 ± 0.11 %; $P < 0.05$).

Anderson et al. (2004) also measured EST in their 4 lines of White Leghorn chickens. Eggshells were dried under a fume hood to a constant weight and measured with the membranes intact using a micrometer. Measurements were taken at 2 different locations on the eggshell, near the mid-line. There were no significant differences in EST between any lines, indicating eggshells were not a focus of selection in the Agriculture Canada breeding program.

Potts et al. (1974) evaluated breaking strength, EST and SG among brown and white eggs. The study consisted of 2 hatches with the first hatch using brown egg strains of Hubbard and Warren and white egg strains of Hyline, Dekalb and Babcock. In the second hatch, the brown strain of Tatum was added, and the white strain of Tatum replaced the Dekalb strain. For hatch 1, 5 to 7 eggs were collected from each of 20 hens after they had been in production for 3 mo (Trial 1) and 7 mo (Trial 2). Measurements were taken on all eggs from each hen and averaged for each trial and the trials pooled. In hatch 2, 50 hens that had been in production for 3 mo contributed eggs for 2 periods of 2 d each. The data collected from the 2 periods were averaged to obtain values for hatch 2.

Eggshell thickness measures among brown layers in both hatches were not different and ranged from 0.322 mm to 0.330 mm ($P > 0.05$). Eggshell thicknesses among the white layers ranged from 0.330 mm to 0.353 mm. The thickest white eggshells came from the Babcock line during the second hatch; this was the only measurement that was significantly different from the others ($P < 0.05$). This study revealed EST differences between white and brown eggs, but minimal differences between strains within a single eggshell color, suggesting little variation in EST among eggs from hens of similar breeds.

As the above studies show, there is little variation among EST measures, specifically between similar breeds. The minimal variation could be attributed to the factors that influence EST, including temperatures over 32°C and low dietary calcium levels. In these studies, temperatures were moderate and diets were constant for all birds. Limited variation among the EST measurements could therefore be expected. While age is also a factor in determining EST, it is possible that the studies cited did not cover a large enough time to notice that effect. In our experiment, the HAS and LAS lines were White Leghorn chickens, which produced white eggs. We suspected little variation in EST between the 2 lines over the 4 mo evaluation period.

Yolk Color. Yolk color is a quality measure in eggs that is quite variable and easily changed. Most consumers in the United States prefer egg yolks that have a light-to-medium color of yellow (Galobart, et al., 2004), while producers that use liquid, frozen, and dried egg products prefer darker YC because the yolks give their products a yellow tint (Zeidler, 2002). The diet of the hen has the greatest influence on YC (Galobart et al., 2004). The YC can be easily manipulated by using synthetic additives, and this is frequently done in many countries. In the United States, however, only naturally occurring products can be used in chicken diets (Zeidler, 2002; Galobart et al., 2004). To achieve the basic yellow color of a typical egg yolk, yellow xanthophylls are needed (Galobart et al., 2004). Because YC is influenced so heavily by the diet, the age and breed of then hen has little influence. Yolk color is subjectively determined by the use of the Roche color fan (Stadelman, 1995; Vuilleumier, 1968).

Popova-Ralcheva et al. (2009) evaluated egg yolks from the hens at 32 and 50 wk of age using the Roche color score. The eggs of the younger hens had a YC score range from 8.20 ± 0.43 to 8.87 ± 0.26, and the YC from eggs from the older hens ranged from 8.21± 0.12 to 8.60 ± 0.28. Because YC is predominantly determined from the feed, and all hens were fed the same

diet, unsurprisingly there were no differences between the YC scores of the different lines and line combinations.

Galobart et al. (2004) considered the effect of saponification of paprika products, marigold products, or both, on the xanthophyll levels in egg YC. Hens were initially fed a wheat-barley diet (white diet) for 15 d to eliminate any reserves of xanthophylls. Experimental diets were then fed for 28 d. In total, 144 laying hens were used at an age of 62 wk. The hens were placed on 1 of 12 dietary treatments (2 replicates of 6 hens each). There were 3 products (SAP, EST-1, and EST-2) included at 4 levels to give 2.25, 4.50, 9.00, and 18.00 mg/kg of red xanthophylls. The SAP came from saponified marigold extract and saponified paprika extract, the EST-1 from marigold meal and gas-extracted paprika oleoresin, and the EST-2 from marigold meal with paprika meal. In all treatments, an adjustment was made to the yellow xanthophylls to 5 ppm. Water and feed was given *ad libitum.* Six to 10 eggs/treatment/d were collected at d 19, 20, 21, 26, 27, and 28 and YC determined using a Roche yolk color fan and by a MiniScan XE HunterLab colorimeter. The YC darkened as the levels of red xanthophylls increased in the diet for all 3 diets for both types of measurement. The SAP diet showed darker levels than either EST diet with values of 7.56, 9.71, 12.39 and 14.31, respectively across the 4 concentration levels ($P < 0.05$).

These 2 studies support that YC is greatly influenced by diet. Popova-Ralcheva et al. (2009) found no difference in YC, which was to be expected in chickens on the same diet. Conversely, in the study by Galobart et al. (2004), a variety of diets were fed, which greatly influenced YC. In our experiment, all hens received the same corn-soybean diet for the length of the analysis period. We did not expect to see a difference in YC among the lines or cycles.

While we expected no change in YC throughout our study, it was beneficial to examine it as a way to monitor consistency in diet.

Egg Quality Summary. Many traits can be evaluated to determine the quality of an egg. Traits range from measurements of exterior SI to interior AH and YC. Traits are influenced by a variety of factors including genetics, hen age and BW, diet and temperature. All traits previously mentioned have been examined under many conditions in multiple studies. Some of the traits described are correlated, and the strength and direction of those correlations will be described in the following section.

Correlations Among Egg Traits

Many egg traits have correlations with other traits. These correlations facilitate examining multiple traits when resources are limited. For instance, correlations between SG with EST and ESW allow shell quality to be determined from SG instead of breaking eggs to measure thickness and weight. This section will address those egg quality traits that have moderate to strong correlations.

Eggshell Traits. The eggshell encompasses the entire egg, and is used in multiple measurements to help determine egg and eggshell quality. Eggshell traits include EST, ESW, SG and eggshell strength among others. Because the eggshell makes up a small portion of the egg, relative to the total weight, eggshell traits may appear to have little importance. Such is not the case. The multiple measurements of the eggshell have correlations with each other, allowing much information to be obtained from these measures. Eggshell strength, for example, is an extremely important eggshell trait, as weak shells may break during shipping. The ability to

examine the strength of the shell through a correlated, non-destructive process, such as SG, would be beneficial.

There is a strong positive correlation between EST and SG. This relationship is plausible, since SG is a way to measure eggshell quality. As the thickness of the eggshell increases, the SG of the egg also increases. A correlation of 0.78 between these 2 traits has been reported (Stadelman;1995). Evaluating eggs of the same color strain, from within the same hatch, the eggs with the thickest shells also have the highest SG scores.

Evidence contradicting a strong positive correlation between EST and SG was reported by Aygun and Yetisir (2010). In this study, both white and brown layers were evaluated post-molt for 40 wk on 4 different diets. A total of 320 Hy-line W-36 and 320 H, and N Brown Nick hens, were used at 57 wk of age. The control group had feed withdrawn for 8 d, followed by a resting diet (13% CP 2500 Kcal kg^{-1} metabolizable energy) for 32 d. The 3 other groups were on a barley (70% Barley, 27% alfalfa), wheat bran (32% wheat bran, 44% corn and 21% alfalfa), or oat (70% oat and 27% alfalfa) based diets fed *ad libitum* for 42 d. On d 43, all hens were placed on the same diet (15% crude protein layer diet).

Eggs that were used in the analysis were collected over wk 76-93 following a molt. At each of the 16 samplings, 160 eggs were analyzed. Among the traits measured were EST and SG; correlations between traits were also calculated. Across all diets and hens, the correlation between EST and SG was 0.06 ± 0.02. This is much lower than the suggestion in Stadelman, (1995) of 0.78. When assessed by diet, Aygun and Yetisir (2010) reported much stronger correlations in the barley and oat-based diets (0.78 ± 0.03 and 0.53 ± 0.03, respectively). The 2 remaining diets had lower estimated correlations of 0.06 ± 0.04 and 0.03 ± 0.04 in the resting

and wheat bran-based diets, respectively. While all diets contained 1% calcium, the wheat-bran based diet had a lower level of dicalcium phosphate, which may have influenced overall shell quality. A reduced level of dicalcium phosphate may have influenced either EST or SG more than the other, causing a reduction in the correlation between the 2 traits.

The correlation between EST and ESW has also been estimated (Stadelman, 1995), and is strong and positive (0.78). This correlation can be explained, in part, by thicker eggshells being heavier.

Zhang et al. (2005) also found a moderately strong, positive, genetic correlation between EST and ESW in dwarf brown-egg layers. At China Agricultural University, a pure-line of brown-egg layers were developed and used in a study to determine correlations among egg quality traits. From this line, 44 sires were selected and each mated to 9 to 10 dams. Eggs were incubated at the same time, hatched on September 1, sexed, pedigreed, wing-banded, and vaccinated against Marek's disease. Chicks were initially kept in an open-side house on constant light. At 2 wk, light was decreased to 22 h. After this, chickens were kept on natural light and transferred to individual cages at 16 wk. The photoperiod was increased by 1 h/d until 17 h of light was reached. At 40 wk, eggs were collected on 3 consecutive d and internal and external quality traits measured. The genetic correlation between EST and ESW was estimated at 0.59. While this value is lower than the value reported by Stadelman (1995) of 0.78, it is still a moderately strong, positive correlation. One of the highest correlations reported by Zhang et al. (2005) was the genetic correlation between EW and ESW, with an estimated value of 0.67.

Interior and Exterior Correlations. Correlations among egg quality traits go beyond eggshell characteristics. Aygun and Yetisir, (2010) examined phenotypic correlations across

many egg traits. The overall strongest relationship recorded was between AH and HU (0.95 ± 0.01). This strong relationship was expected, as the HU is a measure of the AH. Additionally, they estimated correlations of WT with egg width (**EW**) egg length (**EL**) and SG as 0.70 ± 0.01, 0.60 ± 0.02, and -0.32 ± 0.02, respectively. Shell percentage was also correlated with SG (0.39 ± 0.02) and EST (0.37 ± 0.02).

Zhang et al. (2005) also looked at a variety of egg quality traits and found genetic correlations between internal and external traits. They found AH to have positive correlations with WT, SI and ESW (0.32, 0.33, and 0.36, respectively). While these correlations were not as strong as those involving measures of the eggshells, they represented significant relationships among egg traits. These relationships are logical since a "high-quality" egg should be of high-quality for multiple traits. Traits for the compact unit of an individual egg are related to each other in many ways, allowing genetic selection for one or few traits to be beneficial across many traits.

Fertility Among Laying Hens

When a hen is artificially inseminated, sperm is stored in the sperm host glands, which allows her to continue to produce fertile eggs for up to 4 wk after a single insemination (Mauldin, 2002a). According to literature the duration of fertility is typically 7 to14 d (Wishart, 1987; Dunnington et al., 1990). Duration of fertility is influenced predominantly by 2 factors: the number of spermatozoa accepted into the sperm-host gland after insemination and the rate of decline (rate they are released) from the host gland (Wishart, 1987).

Fertility of eggs can be determined by multiple methods. The 3 most common methods are to break fresh eggs, candle eggs that have been incubated for 7 to 12 d, and to break

unhatched eggs on hatch day (Mauldin, 2002b). Breaking out fresh eggs has the advantage of being the quickest way to determine fertility. However, it has drawbacks such as losing potential chicks, assessing only fertility and not other sources of reproductive failure, and a higher level of difficulty in distinguishing fertile and infertile eggs (Mauldin, 2002b).

To measure fertility on fresh eggs, shape, size and color intensity of the germinal disc must all be observed. On a fertilized egg, the blastoderm is typically uniform and symmetrical, and appears as a "donut", whereas an unfertilized egg's blastodisc has jagged edges. The blastoderm is also usually larger than the blastodisc and is less intense in its white color. Delineating fertility through such inspection requires training and practice. In our study, we used this approach, breaking fresh eggs and examining the germinal disc.

Wishart (1987) assessed fertility in eggs, including the number of spermatozoa present in the vitelline membrane, of Rhode Island Red roosters mated to a commercial strain of hens (breed not specified). In the study, roosters and hens were caged individually and fed a commercial diet *ad libitum*. Semen was collected from the roosters and diluted 2, 4, 8 and 16-fold in a glutamate-based diluent. A volume of 40 μl was inseminated into 4 to 8 hens from each of the 4 dilutions. Eggs were obtained from all hens from d 2 to 21 and stored at 5° C. Eggs were broken and the appearance of the germinal disc was assessed.

The following process was used to determine the number of spermatozoa present in the vitelline membrane. The vitelline membrane was sampled (2 to 3 cm^2) near the germinal disc. The sample was rinsed twice in Ca^{2+} and Mg^{2+} free phosphate-buffered saline to remove any yolk residue. The sample was then stained and placed on a glass slide, covered, and sealed. 2 parallel lines (10 mm apart) were drawn down the slide. Using a photomicroscope II, the

comma-shaped sperm nuclei fluoresced blue. The area between the lines was scanned perpendicularly while adjusting up and down to view and count sperm at different depths. Each sample was scanned at different heights 4 to 6 times.

The eggs from each hen were given a fertility status and a number for the spermatozoa on the vitelline membrane. The length of fertility was determined by calculating the median of the number of days after insemination between the first infertile and last fertile egg before 3 consecutive infertile eggs were laid. The logarithm of the number of spermatozoa present in a 5.49 mm^2 area of membrane was strongly correlated (0.61) with the length of the fertile period. The length of the fertile periods of the hens ranged from about 5 to 19 d.

In the study by Dunnington et al. (1990), White Rock lines that were divergently selected for 29 generations for their high and low 8 wk BW and their reciprocal crosses were used to evaluate reproductive fitness. In addition, a control group of White Leghorns that had been selected for their high antibody response to SRBC for 12 generations were used to examine separate effects of sire and dam. All chickens were housed individually and given food and water *ad libitum* while receiving a 14 h photoperiod. At 36 wk, 16 to 20 hens from each of the 5 populations were artificially inseminated using pooled semen from at least 10 roosters from the high weight line. The same procedure was used with another group of 16 to 20 hens, using pooled semen from at least 10 roosters from the low weight line. Samples of hens from the high and low weight lines were also inseminated with pooled semen from at least 10 roosters from the control line. Inseminations were conducted within 10 min of collection and 0.025 mL of undiluted semen was used. Preliminary insemination was done 3 d before the start of the experiment; subsequent inseminations were done on d 1, 8, and 15. Eggs were placed in the incubator on d 8 and 22, thus being set from 1 to 14 d after oviposition. On d 15, all eggs were

candled and eggs with live embryos were transferred to the hatcher. Those that were not transferred were broken and classified as early dead or infertile. Late dead embryos and number of live chicks were recorded at hatch.

In the same experiment, Dunnington et al. (1990) also assessed length of fertility. To determine length of fertility, each day's laid eggs were broken and the germinal disc examined. After 2 consecutive infertile eggs, the hen was classified as infertile. Among all crosses, the average length of fertility ranged from 9.7 to 12.5 d, with the percent fertility ranging from 86.5% to 95.2%. The shortest length of fertility was in high weight line hens mated to the high weight line roosters. The mating of low weight line hens to low weight line roosters resulted in the second highest length of fertility, and the highest percent fertility. These values suggest that resource allocation may play a role in reproductive fitness. Those chickens with heavier 8 wk BW tended to have reduced fertility, suggesting their resources for increased BW are coming from reproduction.

The 2 studies described showed fertility that ranged from 5 to 19 d. We suspected the chickens in our study would fall within this range. We considered fertility independently as a function of the rooster and of the hen. To do so, our LAS and HAS hens were mated to IC males, and our LAS and HAS males were mated to IC hens. This allowed us to see if the length of fertility was more strongly influenced by the hen or by the rooster. This also eliminated confounding of rooster and hen effects on fertility within a line, where mating HAS males to HAS females may have further reduce the length of fertility and mating LAS males to LAS females may have further extend the length of fertility.

Mycoplasma gallisepticum and Associated Problems

Mycoplasma gallisepticum (MG) is a pathogen that is very persistent and highly transmissible spreading horizontally and vertically among chickens and turkeys, as well as other birds (Evans et al., 2005). The disease, which is of respiratory nature, can remain dormant for an extended period of time until the bird undergoes stress. Chronic infections can cause the chickens to enter a catabolic state where they deplete resources attempting to fight the infection which may result in death. Additionally, secondary infections, such as *Escherichia coli*, can further increase mortality within a flock. Symptoms of MG include sneezing, coughing, inflamed and runny eyes, swelling of the sinuses and face, inactivity, and reduction in feed consumption and in egg production (Cutler, 2002; Sainsbury, 1984).

There are currently 3 different live vaccinations for MG based on 3 different strains: F-strain, 6/85-strain, and ts-11 strain. The F-strain, which is a live vaccine resulting in a low infection, provides the highest level of protection, but can cause a potential decrease in egg production, though not as large of a decrease as from a wild strain infection. The F-strain can also result in reduced egg size. Although the 6/85-strain and the ts-11 strain do not cause the decreases in production or egg size, they do not provide as much protection as the F-strain (Leigh et al., 2010).

Research by Gross et al. (1980) showed greater resistance to MG in chickens selected for high antibody response. In their study, resistance to infectious disease was examined in 3 pairs of chicken lines that differed in physiological and immunological properties. The pairs of lines included 2 lines selected for high or low plasma corticosterone response to stress (HPC and LPC), 2 lines selected for high or low antibody response 5 d after an intravenous challenge of

SRBC (HA and LA), and 2 lines that differed in their persistence or lack of persistence in antibody response between 5 and 21 d after a SRBC challenge (PA and NPA).

From hatch until about 3 to 4 wk of age, chicks were kept in flocks of about 40 animals in small electric brooders. They were then moved into Horsfall-Baurer type cages in subdivided flocks of 6 to 8 chickens each. Chickens were given at least 2 wk to acclimate to the new cages. Chickens were challenged with a variety of infectious diseases. The MG challenge involved inoculation via the posterior thoracic air sac, with examinations conducted 14 d later. The scoring system for the number of lesions was: 0 – no lesions, 1 – slight clouding, 2 – slight thickening, 3 – much thickening with extensive exudates. The total score was the sum from the left posterior thoracic and lesser abdominal, left greater abdominal, right posterior thoracic and lesser abdominal, and the right greater abdominal air sacs. The maximum possible lesion score was 16.

The number of chickens with lesions in the LA line (16 of 16) was greater than the number of chickens with lesions in the HA line (9 of 16 chickens). The challenge was repeated and, again, the number of chickens with lesions was greater in the LA line than the HA line with 16 of 32 and 3 of 32, respectively. Even when the challenge was less severe (Gross et al., 1980), the number of chickens with lesions in the NPA line (6 of 18) was greater than the number of chickens in the PA line (0 of 20).

The PA and NPA lines were also challenged 21 d after a subcutaneous vaccination of killed MG. Both lines exhibited lower air sac lesion scores then their non-vaccinated counterparts. It was concluded that the HA line was more resistant to MG than the LA line, as was the PA line compared to the NPA line (Gross et al., 1980). Namely, greater responsiveness

to MG was observed in the line that had a higher antibody response to SRBC and in the line that was persistent in their antibody response.

Due to the contagious nature of MG, the only way to prevent infection of birds in a facility is to empty and sanitize the unit, and vaccinate future birds against MG. We used the results of our testing to determine if the level of infection was greater in either line and if it was adversely affecting egg production and quality. We suspected that our HAS line would be more resistant to MG than our LAS line based on these results.

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Table 1.1 Summary of studies used to examine egg quality traits and fertility.

Year	Authors	Number and type of lines used	Ages	Traits Examined*
1974	Potts et al.	1 Hubbard, 1 Warren, 1 Hyline, 1 Dekalb, 1 Babcock, 1 Tatum	27-46 wk	ST, SG, DF, BS, TN, EW, WL
1987	Wishart	1 Rhode Island Red (males), 1 Commercial Layer (females)	N/A	LF, SN
1990	Dunnington et al.	1 White Rock High (H), 1 White Rock Low (L), 1 White Leghorn	36 wk	ED, LD, LF, F%, HT, HF
1994	Silversides et al.	2 Albino, 2 Non- Albino, 2 Commercial	30-75 wk	EW, YW, SW, AW, AH, HU, Y%, S%, A%
1999	Tharrington et al.	1-1950 base population, 1-1958 base population, 1-1972 base population, 1-1993 commercial strain	28-88 wk	EW, HU, YW, AW, SW, S%, Y%, A%, SP, pH, YF, AS, AP, YS, YP
2002	Ledur et al.	3 pure White Leghorn lines, 6 crosses of the pure lines, 2 Commercial strains	34-65 wk	EW, SG, HU, AH
2003	Monira et al.	1 Barred Plymouth Rock, 1 White Leghorn, 1 Rhode Island Red, 1 White Rock	31-38 wk	EW, EL, EB, SI, BS, AH, HU, ST
2004	Anderson et al.	1-1950 laying hens, 1-1959 4-way Leghorn cross, 1-1972 4-way Leghorn cross, 1-2004 comm. strain	28-86 wk	EW, SW, ST, S%, SF, EL, EB, SI, SG, SA
2004	Silversides and Budgell	1 ISA Brown, 1 Babcock B300, 1 Brown Leghorns	32-68 wk	EW, YW, SW, AW, AH, pH, AV
2004	van den Brand et al.	1 ISA Warren Medium Heavy Layers	25-59 wk	AH, A%, DM%, SI, EW, DMY, Y%, S%, ST, Y/A,
2005	Zhang et al.	1 Pure line brown layers	40 wk	EW, SI, BS, ST, SC, SW, AH, AW, HU, YW, YC
2009	Popova-Ralcheva et al.	1 Combination Red broiler x White Plymouth Rock min, 1 Combination line “C” x White Plymouth Rock mini, 1 Synthetic line Labelle, 1 Combination Red Broiler x Labelle, 1 Line “D” x White Plymouth Rock, 1 Combination synthetic line Labelle	32-50 wk	EW, SI, AW, YW, SW, ST, HU, YC
2010	Aygun and Yetisir	1 Hy-Line W-36, 1 H and N Brown Nick	57-93 wk	EW, SG, AH, HU, ST, S%, EL, EB

*AH=Albumen height, AP=Albumen protein percent, AS=Albumen solids percent, AV=Albumen volume, AW=Albumen weight, A%=Albumen percent, BS=Breaking strength, DF=Deformation, DM%=Dry matter percent, DMY=Dry matter yolk, EB=Egg breadth (width), ED=Early dead embryos, EL=Egg length, EW=Egg weight, F%= Percent fertility of set eggs, HF=Percent hatched of fertile set eggs, HP=Holding period, HT=Percent hatched of total set eggs, HU=Haugh unit, LD=Late dead embryos, LF=Length of fertility, SA=Surface area, SC=Shell color, SF=Shell breaking force, SG=Specific gravity, SI=Shape index, SN=Number of spermatozoa on vitalline membrane, ST=Shell thickness, SW=Shell weight, S%=Shell percent, TN=Shell tint, WL=Width/length ratio, YC=Yolk color, YF=Yolk fat percent, YP=Yolk protein percent, YS=Yolk solid percent, YW=Yolk weight, Y%=Yolk percent, Y/A=Yolk to albumen ratio.

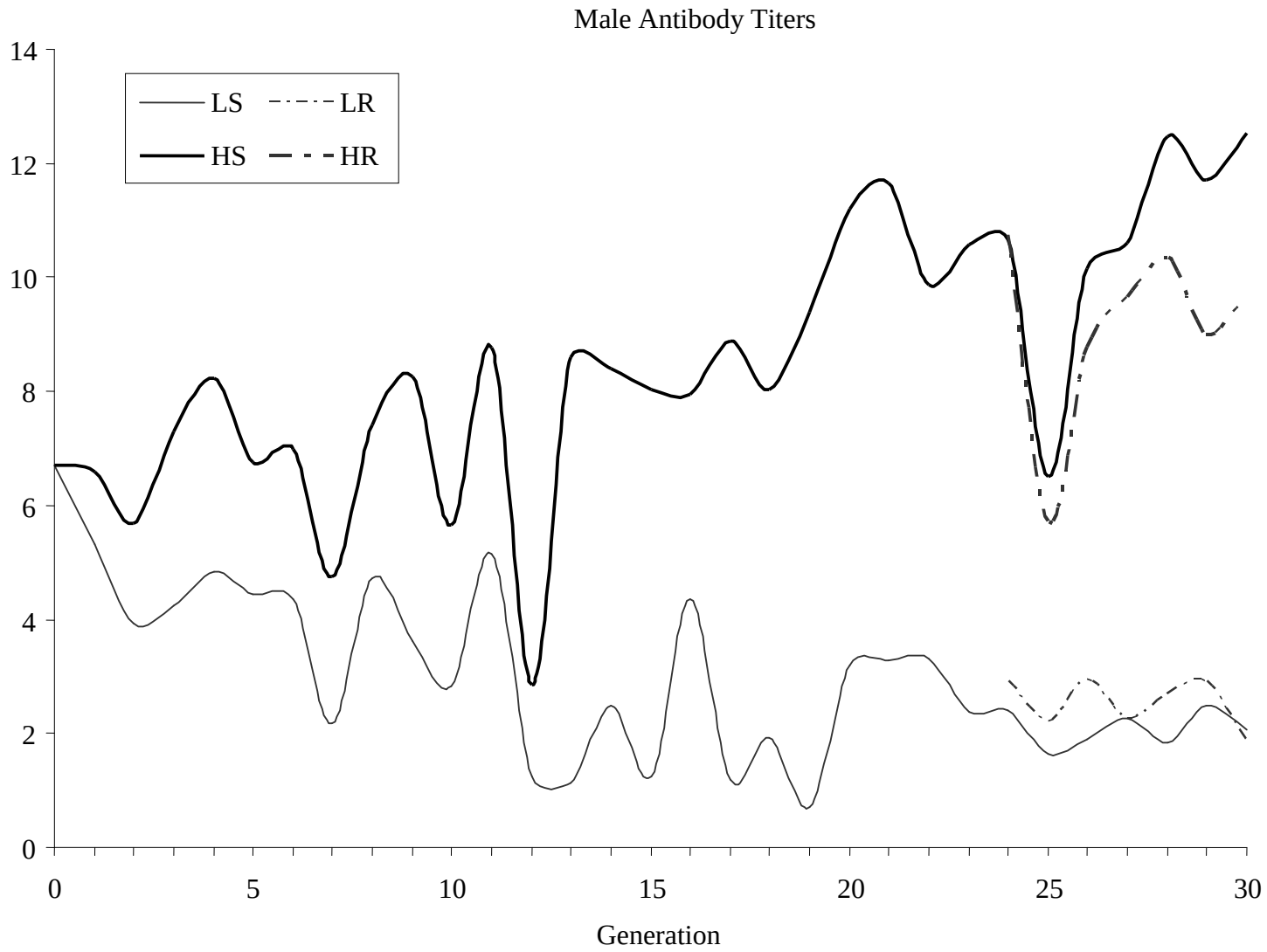

Figure 1.1 Antibody titers of male chickens 5 d after an intravenous injection of 0.1 mL of a 0.25% suspension of sheep red blood cells (SRBC). High (HAS) and low (LAS) titers were selected for 30 generations. At generation 24, relaxed high (HAR) and low (LAR) sublines were initiated at random (Kuehn et al., 2006).

OBJECTIVES

Two lines of White Leghorn chickens selected divergently for 36 generations for high (**HAS**) and low (**LAS**) antibody response to a sheep red blood cell (**SRBC**) challenge were considered. Reproductive soundness and egg quality traits were measured over a 7-mo laying period. Reproductive soundness was determined by commencement of lay parameters and length of fertility. The overall objective of the research was to determine if genetic selection for antibody response, which may affect resource allocations, influences the reproductive soundness of the HAS and LAS lines, and the quality characteristics of the eggs they produce. Parameters of lay commencement included age and BW of the hen at first egg, as well as the weight of the egg. Length of fertility was defined as the total number of days post-insemination until a hen laid 2 consecutive infertile eggs. The egg quality traits considered were egg weight, shape index, shell weight and thickness, and albumin height and yolk color.

HYPOTHESES

i) The LAS hens will commence lay earlier than HAS hens, with heavier first egg weights and at a heavier body weight. Furthermore, the intensity of lay, defined as the ratio of the number of ovulations to the number of d in lay, will be greater in the LAS than HAS line.

ii) The length of fertility will be longer in the LAS than HAS line. Furthermore, the hen will have a larger effect on the length of fertility than the rooster.

iii) No clear expectation as to the impact of selection for antibody response to SRBC on egg quality traits is obvious. However, some egg quality traits will be affected by increasing hen age. Specifically, albumen height will decrease while egg weight will increase.

iv) The prevalence of MG infection will be higher in the LAS than HAS line, which will coincide with lower SRBC titers in the LAS than HAS line. However, there will be no differences in MG or SRBC titers of hens and roosters.

Chapter 2: Reproduction is sounder in chickens selected for low as compared to high antibody response

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ABSTRACT White Leghorn chickens were selected for 36 generations for high (**HAS**) or low (**LAS**) antibody response to SRBC 5 d after an intravenous challenge. The aim of this study was to investigate possible changes in reproductive soundness resulting from that selection. Age and BW at onset of lay (first egg), along with weight of the first egg, were recorded on 45 hens from each line. Intensity of lay was measured as the number of ovulations within a 15 d period over 15 sequential intervals. Three cycles of fertility also were assessed, which coincided with early, middle and late production stages. In order for fertility of males and females within a line to be independently evaluated, roosters and hens were mated by artificial insemination to an unrelated control line. Twenty roosters from each antibody line were considered, as well as the 45 hens. Pooled semen from the control line was used for mating the antibody line hens. Hens from the LAS line commenced lay at a younger age (11.67 ± 3.53 d; $P < 0.001$), at a lighter BW (-169.46 ± 40.20 g; $P < 0.001$), and with greater intensity of lay (2.68 ± 0.25 %; $P = 0.001$) than those from the HAS line. Any differences in intensity thereafter were trivial between lines ($P = 0.42$), with intensity decreasing sharply toward the end of the 7 mo production period in both lines. Length of fertility differed between hens of the antibody lines during the first cycle (3.35 ± .85 d; $P = 0.002$), and between roosters during the first (3.58 ± 1.06 d; $P = 0.02$) and second (3.38 ± 1.07 d; $P = 0.03$) cycles, with the chickens from the LAS line having a longer length of fertility in both sexes. A correlated response in reproductive soundness to divergent selection for antibody response was observed. This may in part be due to differences in resource allocations, with particular impact on duration of fertility.

Key words: chicken, selection, sheep red blood cell, onset of lay, length of fertility

INTRODUCTION

Onset of lay and length of fertility are important aspects of a breeding program in poultry. Lay onset signals that hens have reached sexual maturity, beginning their productive period. With earlier production, hens can be introduced more quickly into breeding programs. Length of fertility, defined as the number of days fertile eggs are produced from a single insemination, helps characterize reproductive soundness in hens. Hens with increased length of fertility have the ability to produce a greater number of fertile eggs after a single mating event, allowing a decrease in the frequency of inseminations.

The duration of fertility after a single insemination is typically between 7 and 14 d (Wishart, 1987; Dunnington et al., 1990). The length of the fertility is affected by both the hen and rooster. Hens influence the number of spermatozoa accepted into the sperm-host gland and the rate at which they are released to fertilize eggs; roosters influence the number of spermatozoa that are present (Wishart, 1987).

Chickens, like other animals, allocate nutritional resources to support maintenance, growth, reproduction, immunity (Siegel and Honaker, 2009) and social interactions. When genetic selection is focused on a specific attribute, resources available for other biological processes are constrained (Gross et al., 2002). For instance, selection for increased antibody response may adversely affect reproductive soundness. When evaluating the efficacy and impact of selection programs, scrutinizing correlated responses to that selection is essential. White Leghorn chickens, which originated from the Cornell Randombred population (King et al., 1959), were selected for high or low antibody response to SRBC. That divergent selection has affected resource allocations, with chickens in the low antibody line reaching sexual maturity at

an earlier age (Siegel et al., 1982; Martin et al., 1990). The objective of this study was to ascertain if, as correlated responses, onset of lay parameters and length of fertility were also affected. We hypothesized that chickens from the low antibody line would have greater reproductive soundness than those from the high antibody line. Reproductive soundness in hens was evaluated by considering their age and BW at the onset of lay, the weight of the first egg, and the intensity of lay. In addition, length of fertility over the first production cycle was assessed in both hens and roosters. We hypothesized that hens from the low antibody line would commence lay at a younger age, with heavier BW and first egg weights, and have a longer duration of fertility.

MATERIALS AND METHODS

The Virginia Tech Institutional Animal Care and Use Committee approved all housing and experimental procedures.

Animals and Housing

Parental Lines. Three lines of chickens were used. Two of the lines were White Leghorn chickens developed at Virginia Tech based on antibody response to a 0.1 ml intravenous injection of a 0.25% solution of SRBC 5 d post injection. Through this selection, low (**LAS**) and high (**HAS**) antibody response lines were developed (Gross et al., 1980; Siegel and Gross, 1980; Martin et al., 1990; Kuehn et al., 2006). In this study, progeny from chickens defining the 36th generation of selection in the antibody lines were used.

In addition, an intercross (**IC**) line was included as a control. The IC line was established from reciprocal crosses of the 41st generation of high and low BW selection lines of White

Plymouth Rock chickens (Siegel, 1962; Dunnington and Siegel, 1996; Márquez et al., 2010). The chickens used in this study were progeny of the 12th generation of the IC line.

Reproduction of Lines. The reproduction of each of the 3 lines consisted of pooling semen from 20 roosters and artificially inseminating (**AI**) 45 hens, and saving eggs from d 2 to 11 post AI. The chicks were hatched December 15, 2009, vaccinated for Marek's disease, and the different lines identified by a toe clip.

Housing. The chicks were initially placed in Petersime cages. Each cage measured approximately 24 cm x 35.5 cm x 99 cm, with 4 cages per row and 6 rows per battery. In total, 3 batteries were used. Seven to 10 chickens from the same line were co-housed in a cage. Lines were stratified among rows across batteries. The first week post-hatch, chicks were kept at between 32° and 38°C. At the second week, temperature was reduced to between 29° and 32°C, with a continued reduction of approximately 3° each week until 7 wk of age. The chicks were offered mashed form feed (CP: 20%, ME: 2,685 kcal/kg as fed) and water *ad libitum*, and kept under constant light. Chicks were weighed individually weekly.

On d 35, chicks were sexed and moved into larger cages. All hens, and 25 randomly selected roosters from each line, were placed in Hartford cages with dimensions of 38 cm x 43 cm x 76 cm. Each battery consisted of 3 rows of cages with 4 cages per row. A total of 5 batteries were used. Five to 7 chickens of the same line were placed in a cage. Hens were housed in 4 batteries, with roosters in the fifth. Lines were stratified among rows across batteries. Combs of the roosters from the LAS and HAS lines were dubbed to minimize injury from the tops of the cages. Lighting was reduced to 14 h/d. A new diet (CP: 14%, ME: 2,827

kcal/kg as fed) and water was offered *ad libitum*, with chickens weighed individually every 3 wk.

At 11 wk, 25 roosters from each line were transferred into individual cages with dimensions of approximately 48 cm x 28 cm x 46 cm. At transfer, all roosters were weighed and wing badged for individual identification. At wk 14, 45 hens from each of the LAS and HAS lines, and 74 hens from the IC line, were transferred using the same procedure. The light schedule remained the same as in the Hartford cages, but the diet changed to a layer ration (CP: 16.1%, ME: 2,752 kcal/kg as fed) offered *ad libitum*. Individual BW was recorded on all chickens every 3 wk until hens commenced lay at approximately 20 wk. In total, 239 chickens were retained.

Experimental Program

Antibody Response to SRBC. In order to confirm line differences, the response of LAS and HAS chickens to a SRBC challenge was determined. This was conducted at the end of the production period using the procedure outlined by Wegmann and Smithies (1966). An injection of 0.1 mL of 0.25% SRBC was given intravenously via the brachial vein. After 5 d, a 3 cc blood sample was obtained from the opposite brachial vein. Endpoint antibody titers were determined using two-fold serial dilutions of serum in a hemagglutination inhibition assay. The endpoint value was obtained by determining the highest dilution at which a positive agglutination response was seen. Titers were expressed as the $\log_2$ of the reciprocal of the dilution (Siegel et al., 1982).

Onset of Lay. The age, BW and egg weight (**WT**), at first egg were recorded on all hens. Age was measured as the number of days post-hatch until a hen's first egg was laid. Thereafter,

daily egg production was monitored and recorded. The IC, LAS and HAS hens commenced lay at on average 163.0 ± 2.0 d, 163.4 ± 2.5 d, and 175.0 ± 2.5 d post-hatch, respectively. An individual hen was considered in lay when she produced 2 normal eggs within a consecutive 10 d period. A line was considered in lay when 80% of the hens in the line were producing. In order to reduce the impact of the difference in physiological age between the LAS and HAS lines, evaluations were staggered accordingly.

Intensity of Lay. Lay intensity was recorded over fifteen 15-d periods starting with the date each hen laid her first egg. Intensity was determined as the number of ovulations divided by the number of days in the period. Periods were 15 d unless a hen died before the end of a period, which was then adjusted accordingly (8 instances). Intensity was not recorded on hens that died or never commenced lay, but was recorded as 0 for hens that ceased production.

Fertility. Fertility was evaluated over the course of 3 cycles for the LAS and HAS lines coinciding with early, middle, and late production. As shown in Figure 2.1, 45 hens from each of the LAS and HAS lines were mated by AI with pooled semen from 20 roosters from the IC line. At each cycle, 20 roosters from each of the LAS and HAS lines were individually mated by AI to 3 hens from the IC line. As an exception, in the third cycle of the HAS line, only 2 IC hens per rooster were still available (in production).

The length of fertility was determined by the number of days that each hen produced fertile eggs. The day of insemination was d 0. Daily, starting with d 2, eggs were collected from the hens within 24 h of being produced and evaluated by breaking to determine fertility. The egg was classified as fertile if the blastoderm was uniform and symmetrical, rather than with jagged edges. The hen was considered fertile until the day she laid her second consecutive infertile egg.

Statistical Analysis

All statistical analyses were conducted using SAS 9.2 (SAS Institute Inc., Cary, NC).

Growth Preceding Onset of Lay. Average BW, and the variance and CV (i.e., SD/mean BW) of BW, at approximately weekly intervals, were analyzed using the GLIMMIX procedure in SAS. The model fitted was:

$$Y_{ij} = \mu + L_i + \beta_{1i}X_{ij} + \beta_{2i}X^2_{ij} + \beta_{3i}X^3_{ij} + e_{ij} \quad [1]$$

where Y_{ij} was the mean, variance or CV of BW of chicken j from antibody line L_i (i=1, …, 3 for lines LAS, HAS and IC, respectively), with X_{ij} the age of chicken j from line i, and $\beta_{1i,}\beta_{2i}$ and β_{3i} the linear, quadratic and cubic regression of age on BW nested within line, and e_{ij} the random error. Two orthogonal contrasts were constructed to compare the values of the regression coefficients among lines. The first was to test differences between the LAS and HAS line, and the second was to test the differences between the average of the two antibody lines and the IC line.

Antibody Response to SRBC. Antibody titers in response to SRBC were analyzed using the MIXED procedure in SAS. The model fitted was:

$$Y_{ijk} = \mu + L_i + S_j + (LS)_{ij} + e_{ijk} \quad [2]$$

where Y_{ijk} was the antibody titer response of chicken k from antibody line L_i (i=1, 2, for lines LAS and HAS, respectively), of sex S_j (j=1, 2, for hens and roosters, respectively), with $(LS)_{ij}$ the interaction of line and sex, and e_{ijk} the random error.

Onset of Lay. Three measures associated with onset of lay were evaluated: age, BW and WT at first egg. Data on one IC hen were excluded because her onset of lay was 40 d after all contemporaries.

The GLIMMIX procedure was used to analyze age, BW and WT at first egg using the model:

$$Y_{ij} = \mu + L_i + e_{ij} \quad [3]$$

where Y_{ij} was the trait of interest for chicken j of line L_i (i=1, …, 3 for lines LAS, HAS and IC, respectively) and e_{ij} was the random error.

As an additional analysis of WT, the GLIMMIX procedure again was used adding BW at first egg as a covariate to model [3]. The model fitted was:

$$Y_{ij} = \mu + L_i + \beta_i X_{ij} + e_{ij} \quad [4]$$

where Y_{ij} was the WT of chicken j of line L_i (i=1, …, 3 for lines LAS, HAS and IC, respectively), with X_j the BW of chicken j from line i at first egg, and β_i the linear regression of BW on WT nested within line.

In the analyses of commencement of lay, two orthogonal contrasts were constructed to compare line means. As before, the first was to test the difference in WT between the LAS and HAS line, and the second was to test the difference between the average of the two antibody lines and the IC line. In addition, correlation coefficients were obtained for pair wise combinations of age, BW and WT within line. The CORR procedure of SAS was used.

Intensity of Lay. Since intensities were obtained as the ratio of number of ovulations to number of days in a period, they formed binomial proportions. Due to the distributional

properties of proportions, an angular transformation was used with the transformed values obtained as the arcsine of the square root of the intensity multiplied by 180/π. These data were analyzed with the GLIMMIX procedure of SAS using the model:

$$Y_{ijk} = \mu + L_i + P_j + (LP)_{ij} + e_{ijk} \quad [5]$$

where Y_{ijk} was the transformed intensity of chicken k from line L_i (i=1, …, 3 for lines IC, LAS and HAS, respectively), for period P_j (j=1, …, 15), with $(LP)_{ij}$ the interaction of line and period, and e_{ijk} the random error. Hen nested within line was the experimental unit, with period considered a repeated measure. An auto-regressive covariance structure among periods with homogenous variances was assumed. This covariance structure also assumed equal spacing and non-equal correlations between periods.

Least squares means were derived for the interaction of line and period. The means were separated by period using the slice procedure in SAS, and compared using the adjusted Tukey-Kramer test. Differences in intensity between the LAS and HAS lines, and between the average of the selections lines and the IC line, were tested using orthogonal contrasts. Means and standard errors were back-transformed to the original scale for reporting.

Fertility. Fertility was analyzed separately for hens and roosters. The hen fertility data were analyzed using the GLIMMIX procedure of SAS fitting the model:

$$Y_{ijk} = \mu + L_i + C_j + (LC)_{ij} + e_{ijk} \quad [6]$$

where Y_{ijk} was the number of days of fertile egg production from hen k from antibody line L_i (i=1 or 2 for lines LAS and HAS, respectively), and cycle C_j (j=1, …, 3), with $(LC)_{ij}$ the interaction of line and cycle, and e_{ijk} the random error. Hen nested within the interaction of line and cycle was

random and the experimental unit, with cycle considered a repeated measure. A compound symmetry covariance structure among cycles with homogenous variances was assumed. This covariance structure also assumed equal correlations and non-equal spacing between cycles.

Orthogonal contrasts were constructed to test for differences in fertility between the antibody lines, between the first and second cycle, and between the average of the early cycles (average of the first and second cycles) and the late (third) cycle. Two additional orthogonal contrasts were constructed to test for presence of interactions: that between line and cycle for the first two cycles, and that between line and the average of the early cycles and the late cycle.

The rooster fertility data were analyzed using the GLIMMIX procedure of SAS fitting the model:

$$Y_{ijkl} = \mu + L_i + C_j + R(L)_{ki} + (LC)_{ij} + e_{ijkl} \quad [7]$$

where Y_{ijkl} was the number of days of fertile egg production from hen l mated to a rooster from antibody line L_i (i=1 or 2 for lines LAS and HAS, respectively), in cycle C_j (j=1, …, 3), mated to rooster R_k (k=1, …, 34), with $(LC)_{ij}$ the interaction of line and cycle, and e_{ijk} the random error. Rooster nested within the interaction of line and cycle was random and the experimental unit, while cycle was considered a repeated measure. Again, a compound symmetry covariance structure among cycles with homogenous variances was assumed. This structure also assumed equal correlations and non-equal spacing between cycles. The same orthogonal contrasts were fitted as with hen fertility.

RESULTS

Growth Preceding Onset of Lay. Least squares means for BW for all 3 lines were recorded weekly from hatch until 5 wk of age, then every 3 wk until commencement of lay at around 21 wk. Changes in BW during these weeks are shown in Figure 2.2. In all lines, increases in BW followed a sigmoidal pattern. Chickens in the antibody lines had similar average BW each week. However, chickens in the IC line grew to much heavier weights, with a demarcation in BW from the antibody lines starting at 9 wk of age. In the fit of the polynomial (cubic) regression, values of the regression coefficients were similar between antibody lines for the linear, quadratic and cubic coefficients ($P > 0.37$). The values of these regression coefficients in the IC line differed from the average of the values in the antibody lines for the quadratic and cubic terms ($P < 0.003$).

Variability among BW each week was similar in the two antibody lines, and much less than in the IC line (Figure 2.3). This was to be expected. The BW in the antibody lines reflected 36 generations of within-line selection, while the BW in the IC line reflected a reciprocal cross that was randomly bred (with the exception of mating full sibs) thereafter. In the fit of the polynomial (cubic) regression, values of the regression coefficients were similar between antibody lines for the linear and cubic terms ($P > 0.51$). However, the coefficients in the IC line differed from the average of the antibody lines for the linear, quadratic and cubic terms ($P < 0.001$).

In all lines, the variance increased with mean BW due to scaling. Still, when expressed as a coefficient of variation, variability in the IC line remained appreciably higher. The values of the regression coefficients were similar between the antibody lines for all 3 terms ($P > 0.83$) yet

differed when comparing the IC line to the average of the antibody lines (linear: $P = 0.001$; quadratic: $P = 0.01$; cubic: $P = 0.06$).

Antibody Response to SRBC. Least squares means for antibody titers (log 2) in response to an intravenous SRBC challenge for the LAS hens and roosters were 3.10 ± 0.35 and 3.42 ± 0.52, respectively; for the HAS hens and roosters, the titers were 9.91± 0.34 and 10.23 ± 0.48, respectively. Antibody titers differed substantially between the HAS and LAS lines (3-fold difference; $P < 0.001$) but not sexes ($P = 0.45$), and there was no interaction of line and sex ($P = 0.99$).

Onset of Lay. Least squares means for age, BW and WT at commencement of lay for the 3 lines are reported in Table 2.1. Age at onset of lay in the LAS line was approximately 11.67 ± 3.5 d earlier than in the HAS line ($P = 0.002$). The average age at onset of lay in the antibody lines was 6.16 ± 2.64 d later than in the IC line ($P = 0.02$).

At first egg, HAS hens weighed 169 ± 40 g more than LAS hens ($P < 0.001$). Furthermore, the average BW from the hens of both antibody lines was 476 ± 30 g less than the IC line ($P < 0.001$).

The first WT from the LAS line was not different from that of the HAS line (a difference of 1.28 ± 0.78 g/g; $P = 0.10$). However the average WT of the antibody lines was 4.41 ± 0.58 g heavier than the IC line ($P < 0.001$).

The BW of the hen at first egg was added to the model (model [3]) as a covariate (model [4]). Egg weight increased with BW for the LAS and HAS lines, but there was no effect of BW on WT in the IC line. The slope of the regression of WT on BW was 0.013 ± 0.004 g/g ($P = 0.003$) for the LAS line and 0.011 ± 0.004 g/g ($P = 0.007$) for the HAS line; however, in the IC

line, the estimate of the regression coefficient did not differ from zero (0.001 ± 0.002 g/g; P = 0.64). Once BW was accounted for, the difference in the weight of first egg between the LAS and HAS lines increased although it remained small (1.73 ± 8.73; P = 0.84). The average WT of antibody lines, once adjusted for BW, was 11.7 ± 5.5 g heavier than the IC line (P = 0.04).

Age at onset of lay and WT were moderately and positively correlated within each line (IC: 0.403; LAS: 0.412; HAS: 0.403; $P < 0.008$), while there was no correlation between age and BW at first egg in any line ($P > 0.07$). The WT and BW were positively correlated within the antibody lines (LAS: 0.421; HAS: 0.360; $P < 0.018$) but not in the IC line (P = 0.61).

Intensity of Lay. Results for intensity of lay by line are shown in Figure 2.4 across the 15 periods. While there were no overall differences in intensity between the antibody lines (P = 0.42), the average intensity of lay in the LAS and HAS lines (43.85 ± 0.03 %) was greater than in the IC line (37.13 ± 0.02 %; $P < 0.001$).

Intensity at the start of production (period 1) was lower for HAS hens than LAS (P = 0.001) and IC (P = 0.04) hens, based on testing slices of the interaction of line and period. The intensity of lay decreased much earlier in the IC as compared to antibody lines. Specifically, intensity was lower in the IC line in periods 8 through 12 ($P < 0.02$) when compared to the LAS line, and in periods 7 through 12 ($P < 0.009$) when compared to the HAS line. At periods 13 and 14, the IC line had slightly greater intensity than the HAS line ($P < 0.02$). Intensity in the antibody lines also differed at period 13 (P = 0.006). However, at this late stage, production levels were quite low in all lines

Fertility. Least squares means for length of fertility in hens by line are shown in Figure 2.5 across cycles. When comparing the means for the antibody lines, a difference was only

detected for cycle 1 (LAS: 9.60 ± 0.59 d; HAS: 6.25 ± 0.62 d; $P = 0.002$). A tendency for longer fertility in the LAS than the HAS line persisted over the subsequent cycles (Cycle 2: 1.56 ± 0.85 d, $P = 0.45$; Cycle 3: 2.38 ± 0.85 d, $P = 0.06$), although the differences were not significant. Still, fertility in hens in the LAS line was longer overall (9.58 ± 0.39 d) than in hens in the HAS line (7.15 ± 0.39 d; $P < 0.001$). There was no overall effect of cycle ($P = 0.12$) on fertility in hens. However, the average length of fertility of both antibody lines over cycles 1 and 2 was 0.98 ± 0.49 d shorter than the duration in cycle 3 ($P = 0.04$). There were no interactions among lines and cycles on fertility in hens ($P > 0.10$).

Least squares means for length of fertility in roosters by line are shown in Figure 2.6 across cycles. The LAS line roosters were fertile for 8.25 ± 0.47 d, while the HAS line roosters were only fertile for 5.14 ± 0.54 d ($P < 0.001$). There was also a difference between the antibody lines during cycles 1 and 2. The LAS line was fertile 3.58 ± 1.06 d ($P = 0.02$) longer in cycle 1, and 3.38 ± 1.07 d ($P = 0.03$) longer in cycle 2. During cycle 3, the difference between the LAS and HAS line was less (2.36 ± 1.2 d; $P = 0.34$). Among roosters, there were no effects of cycle on fertility when comparing the first to the second cycle ($P = 0.06$) or when comparing the average of the early to the late cycle ($P = 0.86$). As with hens, there also was no line by cycle interactions for fertility in roosters ($P > 0.38$).

DISCUSSION

Antibody Response to SRBC. There were clear differences in antibody response to SRBC between the antibody lines, with an approximate three-fold difference between the lines. The average titer value for the LAS line was low (3.26 ± 0.31), as expected, with that of HAS line considerably higher (10.07 ± 0.29). Still, the titer value in the HAS line was lower than in

earlier generations of this line. As of the 34th generation, the average titer value for the HAS line was greater than 14 (Siegel and Honaker, 2009). Age and environmental factors may have affected titer values, specifically temperature and differences in the donor sheep used when harvesting SRBC (Siegel and Honaker, 2009). The chickens used in this study were hatched approximately 9 mo later than the parental lines, with antibody titers assessed in October rather than April. Perhaps more importantly, the testing was conducted when the chickens were adults rather than juveniles. Furthermore, with long-term selection, "waves of response" may be observed as reported previously in these lines. In generations 11 and 25, average antibody response in the HAS line decreased dramatically followed by sharp increases in the subsequent generation (Kuehn et al., 2006). The lower antibody titer in the HAS line observed in this study simply may reflect this oscillatory pattern.

Onset of Lay. Attributes measured at the onset of lay differed among the three lines. The LAS line commenced lay at the same time as the IC line, but both lines came into lay approximately 12 d earlier than the HAS line. This is consistent with previous examination of the antibody lines: in generations 10, 14 and 36 (parents of the chickens used in the current study) the LAS line commenced lay 13, 22 and 13 d earlier than the HAS line, respectively (Martin et al., 1990; Siegel, unpublished). In a different selection experiment involving White Rock chickens selected for BW at 8 wk of age, those selected for low BW commenced lay later than those selected for high BW (Anthony et al., 1989). Dunnington and Siegel (1996) observed similar results when examining White Plymouth Rock chickens also selected for their 8 wk BW. Such line differences in age at sexual maturity suggest a correlated response to the specific selection regime.

The average age of LAS hens at the start of production in this study was similar to that observed in this line in earlier generations. However, the onset of lay in the HAS line was 8 and 11 d earlier, respectively, than in generations 10 and 14 (Martin et al., 1990), but only 2 d earlier than in generation 36 (Siegel, unpublished). The younger age of sexual maturity in the HAS line compared to early generations may be due to a reduction in threshold for BW required for onset of lay, and may be seen as a correlated response to the selection for high antibody response to SRBC. In a study examining sexual maturity in dwarf and normal chickens selected for high and low 8 wk BW, younger age-at-lay onset was observed in the high weight line non-dwarf hens (Brody et al., 1983). With selection for high weight, non-dwarf hens reached the BW threshold to begin egg production sooner than in their low weight counterparts.

The LAS line in the current study began production at a lighter BW than both the HAS and IC lines. The IC hens had a much heavier average BW at first egg, but this simply reflects breed differences (White Plymouth Rock vs. White Leghorn). The average BW of hens at sexual maturity in the LAS line was similar to that of hens from generations 11 and 14. However, hens from the HAS line were 96 and 160 g heavier at commencement of lay when compared to HAS hens in generations 11 and 14, respectively (Martin et al., 1990). Conversely, the average BW in the parents of the hens in the current study from both antibody lines at onset of lay was considerably less than in generations 11 and 14 (Siegel, unpublished). This decrease suggests a lower BW threshold requirement, over time, for hens to begin production.

The increase in average BW in the hens used in this study at onset of lay, without an increase in age, as compared to their parents suggests a clear environmental impact of the change in hatch date. These increased gains were achieved even though the feed offered was the same. The resources needed for a hen to commence lay apparently were not countered by the selection

for increased antibody response to SRBC. While onset of lay can be influenced by genetic selection, minimum standards must be met by each hen in regards to age, BW and carcass composition (Dunnington et al., 1984).

The weight of the first egg did not differ between the HAS and LAS lines, though both antibody lines had heavier eggs than the IC line. This difference is likely explained by breed differences. The HAS and LAS hens are White Leghorn chickens, while the IC hens are White Plymouth Rock chickens. Measures such as first WT are line specific, so differences between them are reasonable. Differences in WT among other breeds have been reported (Monira et al., 2003; Popova-Ralcheva et al., 2009). Noteworthy was the relationship between WT and BW in the antibody lines. The HAS and LAS lines had a significant relationship between WT and BW, while no such relationship existed in the IC line. This lack of relationship in the IC line is due, in part, to the large range of BW, and small range of WT, at first egg when compared to the antibody lines.

Intensity of Lay. Intensity of lay was greater in the LAS than HAS line only during the first period and once again during period 13 when production had almost reached completion. It was hypothesized that the HAS line would have decreased intensity compared to the LAS line because of resources allocated to enhance antibody response. Such was the case in generation 14 (Martin et al., 1990). The lack of difference in intensity between the HAS and LAS lines in the current study suggest sufficient resources were available for egg production, regardless of differences in their antibody response to SRBC.

The antibody lines differed from the IC line by having a higher intensity of lay during mid-production. Although intensity began to decrease in the IC line at period 6, intensities were

still increasing in the antibody lines. By period 7 and 8 in the HAS and LAS lines, respectively, the antibody lines had a more intense rate of production compared to the IC line, which persisted through period 12. This decrease in intensity in the IC line occurred gradually over time. In the antibody lines, intensity remained fairly stable until late production, followed by a sharp drop around period 12. The IC line may be more susceptible to the ageing process, causing the hens to limit intensity throughout the course of their production.

Fertility. The length of fertility was different between the antibody lines for both hens and roosters, with the LAS line having a longer duration of fertility. However, differences were only found for the first two cycles among roosters and the first cycle among hens.

Variation in length of fertility is due to the contribution of both the hen and rooster (Wolc et al., 2009). According to Wishart (1987), duration of fertility is influenced predominantly by the number of spermatozoa that are accepted into the sperm-host gland, post insemination and the rate at which they are released. It has also been hypothesized that as a hen ages, there is decreased efficiency of sperm storage (Gumulka and Kapkowska, 2005). These factors, combined with resource allocation, may help to explain the initial differences in the LAS and HAS lines, and why they did not persist. The initial difference in fertility between the lines may be due to reproductive resources in both hens and roosters being reduced due to selection for high antibody response. As the animals age, however, the reduction in efficiency of sperm storage may mask the line difference.

The overall average length of fertility was longer in hens as compared to roosters in both lines. The increased length of fertility in hens suggests that hens may have a greater influence on the success of fertilization, than do roosters. Conversely, heterosis when mating the antibody

and IC lines may be responsible for this difference. There may be a difference in fertility between hens and roosters of the IC line, which was not examined, that may have been amplified with mating to the different antibody lines.

Conclusions. Reproductive soundness, as a whole, appears greater in the LAS line as compared to the HAS line. The LAS hens were able to begin production at an earlier age and lighter BW. These hens also entered lay at an increased intensity compared to their HAS counterparts. Lastly, the LAS line was able to maintain longer lengths of fertility earlier in production, suggesting more efficient storage within the sperm host gland. Further research is needed to understand the difference between sexes and their independent effects on length of fertility. Mating roosters and hens from the antibody lines to several different control lines would allow differences between sexes to be more easily seen, particularly if heterotic effects on reproductive soundness differ depending on the breed combination.

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Table 2.1 Least squares means for commencement of lay aspects across line [intercross (IC), low antibody select (LAS) and high antibody select (HAS) response lines]

	Commencement of Lay Aspects		
Line	Age (d)	Body Weight (g)	Egg Weight* (g)
IC	163.0[b]	1949.5[a]	34.9[b]
LAS	163.4[b]	1388.6[c]	39.9[a]
HAS	175.0[a]	1558.1[b]	38.7[a]
Maximum SEM	2.5	28.6	0.8

[abc]Means in the same column with different superscripts are different ($P < 0.05$).

*Covariate of body weight was not included when calculating means for egg weight.

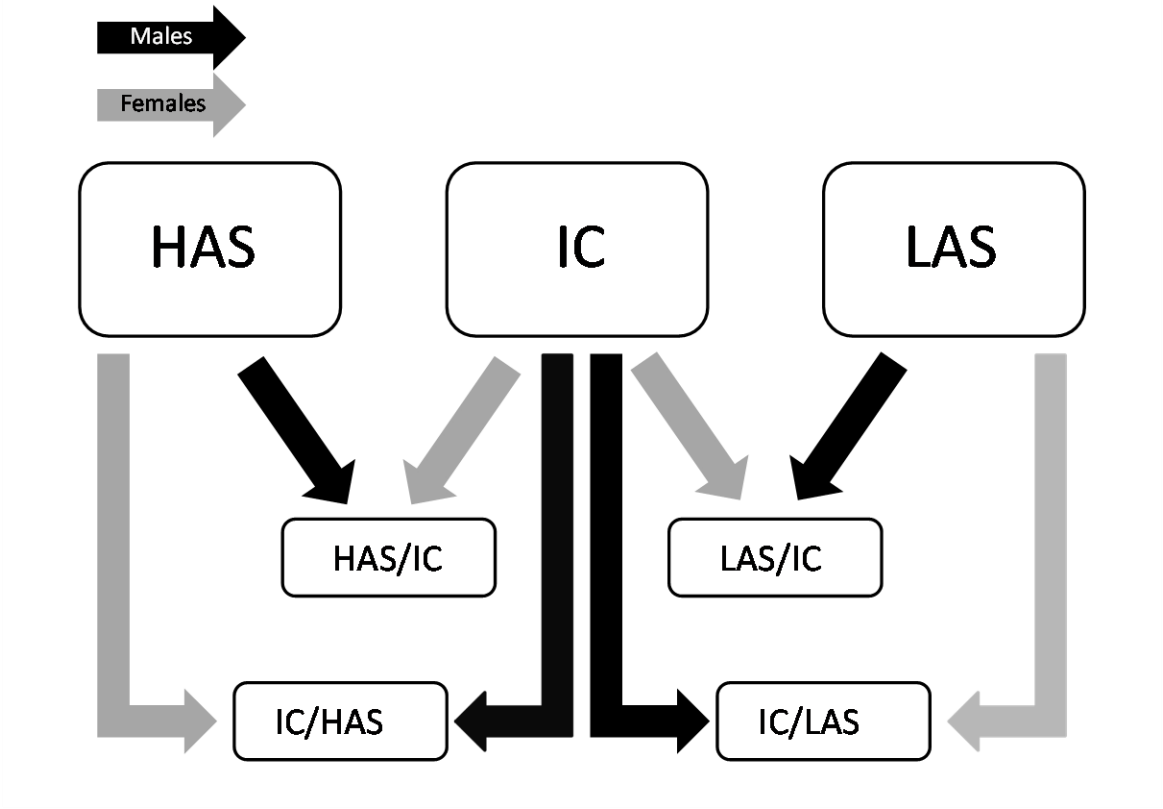

Figure 2.1 Schematic of mating design for low (LAS) and high (HAS) antibody select lines with the intercross (IC) line.

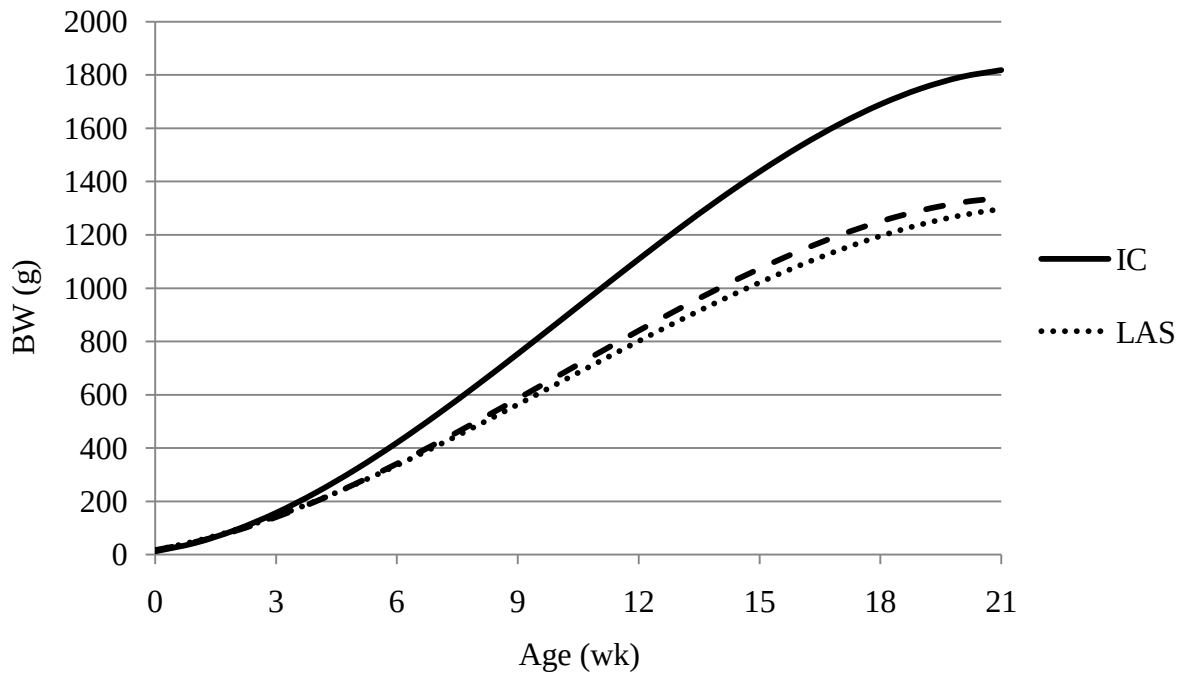

Figure 2.2 Mean BW through 21 wk of age by line and week [intercross (IC), low antibody select (LAS) and high antibody select (HAS) response lines].

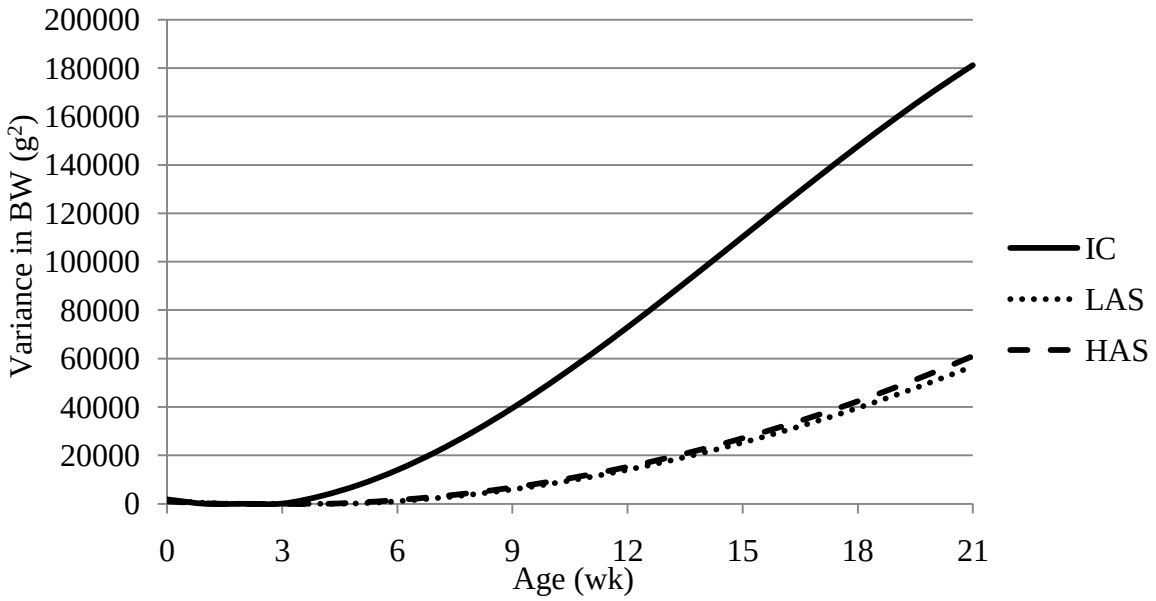

Figure 2.3 Variance in BW through 21 wk of age by line and week [intercross (IC), low antibody select (LAS) and high antibody select (HAS) response lines].

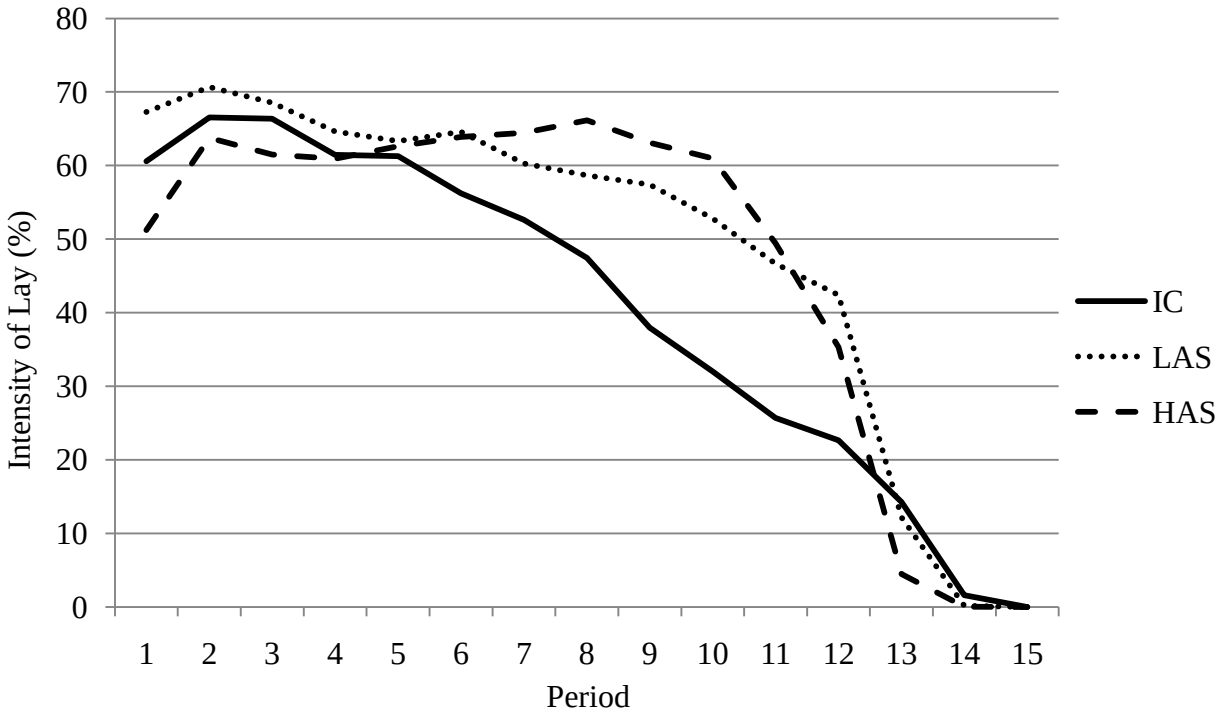

Figure 2.4 Mean intensity of lay across period by line [intercross (IC), low antibody select (LAS) and high antibody select (HAS) response lines]. When lines compared, only lay intensity at periods 1 and 13 differed between the LAS and HAS lines ($P < 0.006$). However the IC line had decreased intensity compared to the LAS line in periods 8 through 12 ($P < 0.009$) and the HAS line in periods 7 through 12 ($P < 0.04$). Briefly, in periods 13 and 14, the IC line had greater intensity of lay compared to the HAS line ($P < 0.02$).

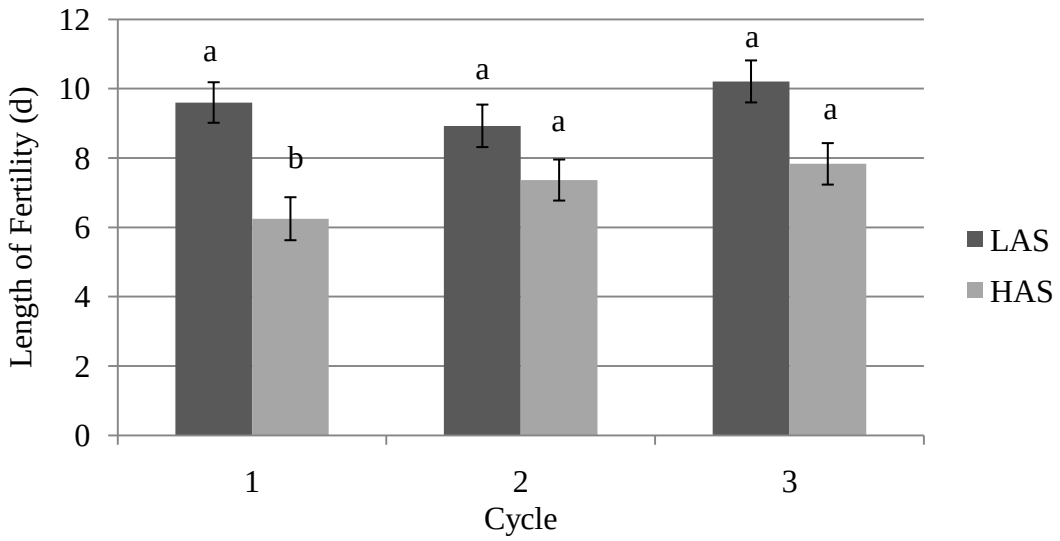

Figure 2.5 Length of fertility of females across cycles by line [low antibody select (LAS) and high antibody select (HAS) response lines]
[ab]Means within a cycle with different superscripts are different ($P < 0.05$).

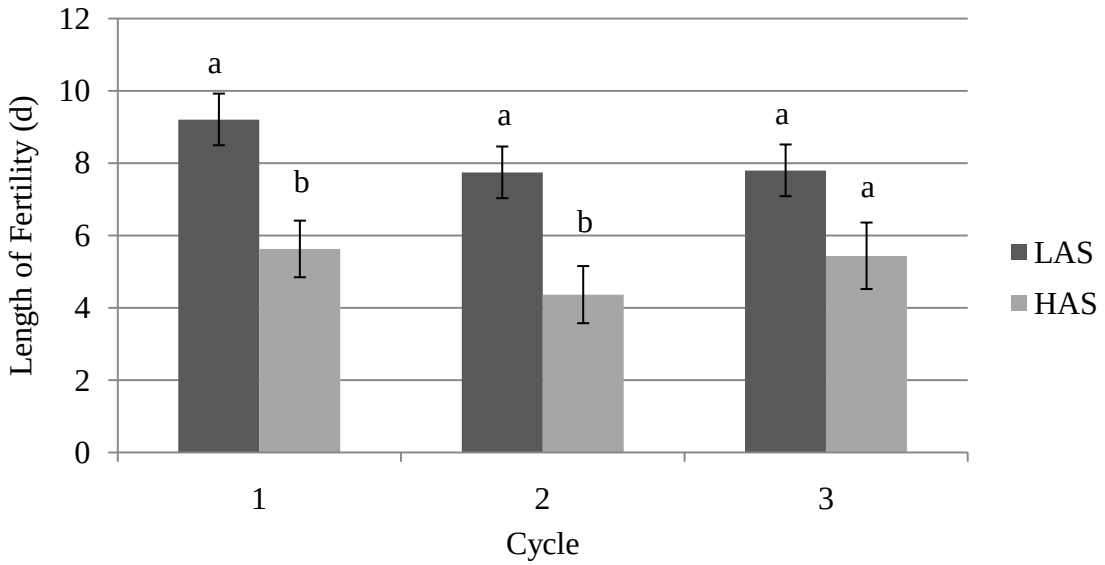

Figure 2.6 Length of fertility of males across cycles by line [low antibody select (LAS) and high antibody select (HAS) response lines]
[ab]Means within a cycle with different superscripts are different ($P < 0.05$).

Chapter 3: Egg Quality Traits are Superior in Hens Selected for High as Compared to Low Antibody Response

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ABSTRACT White Leghorn chickens were selected for 36 generations for high (**HAS**) or low (**LAS**) antibody response to SRBC 5 d after an intravenous challenge. This study's objective was to determine differences in egg quality and response to *Mycoplasma gallisepticum* (**MG**) resulting from that selection. In total, eggs from 45 hens from both lines were assessed for shape index (**SI**), weight (**WT**, g), albumen height (**AH**, mm), Haugh units (**HU**), yolk color (**YC**), and eggshell weight (**ESW**, g) and thickness (**EST**, mm). Three cycles representing early, middle and late stages of production were examined. Blood samples were collected from hens, and 25 roosters per line, at the start and end of production to evaluate MG antibody response by ELISA. Eggs from HAS hens had higher SI scores (4.12 ± 0.55; $P < 0.001$) and larger AH (0.27 ± 0.12 $P < 0.001$) and HU (1.89 ± 0. 91; $P = 0.04$) measures than LAS hens; conversely, eggs from LAS hens had greater EST (0.03 ± 0.01 g; $P < 0.001$) and heavier ESW (0.66 ± 0.09 g; $P < 0.001$) than HAS hens. Lines were similar for WT and YC ($P > 0.52$). Albumen height and HU decreased ($P < 0.001$), while WT, ESW and EST increased ($P < 0.001$), over cycles for both lines. However, SI decreased in LAS hens, yet increased in HAS hens, across cycles ($P < 0.001$). An interaction between line and cycle was observed in WT, SI, ESW and EST ($P < 0.001$), but only for WT did the interaction cause re-ranking across cycles. The HAS line had greater MG antibody response than the LAS line (111.6 ± 16.1; $P < 0.001$). Furthermore, antibody response was greater at the second than the first sampling (107.0 ± 12.5; $P < 0.001$), and in roosters than hens (77.4 ± 12.7 g; $P < 0.001$). Egg quality was, generally, superior in HAS hens, suggesting selection for increased antibody response does not jeopardize egg quality.

Key words: chicken, selection, sheep red blood cell, egg quality, *Mycoplasma gallisepticum*

INTRODUCTION

The definition of quality in a chicken egg depends on the point of reference. That is, the key attributes of quality may differ for the chicken, the poultry breeder and processor, and the consumer. For the egg industry, quality is determined by egg shape, weight, albumen height, yolk color, and shell attributes. For some of these measures, target values or ranges are prescribed. Egg shape and eggshell thickness are two examples where specific shapes and thicknesses are considered ideal. Eggs that are too narrow or too round will not fit properly into pre-made packaging, and sharp eggs are not as resistant to damage during handling as normal shaped eggs (Anderson et al., 2004; Altuntas and Sekeroglu, 2007). Furthermore, eggshells that are too thin may break easily, while eggshells that are too thick may cause difficulties with automatic breakers. For other traits, such as yolk color, preferences depend on the customer and vary widely globally (Galobart, et al., 2004).

Egg quality traits are influenced by genetics, the age and BW of the hen, diet and temperature (Silversides, 1994; Tharrington et al., 1999; Silversides and Budgell, 2004; van den Brand, 2004). Thus genetic selection, and management, can be used to modify egg quality to fit commercial requirements.

Mycoplasma gallisepticum (**MG**) is a highly transmissible pathogen which can adversely affect laying hens as well as other birds (Evans et al., 2005; Leigh et al., 2010). Its symptoms, which are primarily respiratory, can result in decreased egg production; if the infection is severe, it can cause a complete cessation in production (Evans et al., 2005; Leigh et al., 2010). While there is considerable literature on the effects of MG on egg production (Carpenter et al., 1981;

Burnham et al., 2002), little is available explaining its possible negative effects on egg quality traits.

The objective of this study was to determine if genetic selection for antibody response to sheep red blood cells (**SRBC**) in White Leghorn chickens (Siegel and Gross, 1980; Kuehn et al., 2006), which affects resource allocation (Siegel et al., 1982, Martin et al., 1990, Gross et al, 2002), influenced egg quality traits and resistance to MG. Nutritional resources are typically allocated to maintenance, growth, reproduction, immunity (Siegel and Honaker, 2009) and social interactions. When selection is conducted for a specific criterion, such as antibody response, resources available to support other physiological functions may be limited (Gross et al., 2002). Thus, although immunocompetence may be improved through selection for higher antibody response, egg quality may suffer.

In this study, egg traits were evaluated over the course of three cycles representing early, middle and late production in hens from lines selected for low (**LAS**) and high (**HAS**) antibody response to a SRBC challenge. The chickens were also evaluated to determine seroprevalence to MG exposure at the start and end of their production period.

MATERIALS AND METHODS

Animals and Housing

The chickens used in this study, and their housing is outlined in chapter 2.

Experimental Program

Evaluation. Egg traits of the LAS and HAS lines were evaluated over 3 cycles. Within each cycle, from a period lasting 4 to 7 d, 2 eggs were collected from each hen. Typically, eggs

laid on d 1 and 3 of the cycle were assessed. When a hen did not produce an egg on d 1 or 3, where possible, one was collected the subsequent day. Measurements from the 2 eggs within a cycle were averaged.

Egg Quality. Quality traits were evaluated for each egg. Eggs were weighed individually, and their egg weight (**WT**) recorded to the nearest 0.1 g. Egg length (**EL**) and width (**EW**) was measured to the nearest 0.1 cm using calipers. The EW was divided by the EL and multiplied by 100 (van den Brand et al., 2004) to obtain the shape index (**SI**). Prior to the start of the study, the consistency of these measures were tested. The correlation between pairs of width measurements was 0.998, whilst that between pairs of length measurements was 0.999 (data not shown).

Eggs were broken onto a flat surface and the height of the albumen (**AH**) measured in mm using a tripod micrometer halfway between the edge of the yolk and thick albumen. The color of the unbroken yolk (**YC**) was determined using a Roche color fan (Vuilleumier, 1968). The AH measurement was combined with WT to obtain Haugh units (**HU**) (Haugh, 1937).

Eggshells, with outer membranes intact, were placed upside down in a flat to drain and dry overnight. They were then weighed, with the eggshell weight (**ESW**) recorded to the nearest 0.01 g. The thickness of the eggshell (**EST**) was measured in 3 places around the midline to the nearest 0.01 mm, which were averaged. Additionally, the variance of the 3 thickness measurements was obtained. The consistency of thickness measures was assessed at the start of the study. Two sets of 3 thickness measurements were averaged, and those averages correlated. The correlation coefficient was 0.931.

Mycoplasma gallisepticum. There is historic evidence of an MG infection at the facility where the chickens were housed, which may have affected productivity (Leigh et al., 2010). Blood samples were collected before the first, and after the third, cycle from both roosters and hens. Prevalence of MG antibody in serum was determined using an ELISA kit (IDEXX laboratories, Inc., Westbrook, ME).

Statistical Analysis

All analyses were conducted using SAS 9.2 (SAS Institute Inc., Cary, NC).

Egg Quality. Nine measures associated with egg quality attributes were evaluated. These characterized egg shape (EL, EW and SI), weights (WT and ESW) and quality (EST, AH, HU and YC), and were measured over the course of 3 cycles.

The GLIMMIX procedure in SAS was used to analyze all egg traits. The following model was fitted:

$$Y_{ijk} = \mu + L_i + C_j + (LC)_{ij} + e_{ijk} \quad [1]$$

where Y_{ijk} was the trait of interest for chicken k of antibody line L_i (i=1 and 2 for lines LAS, HAS, respectively), from cycle C_j (j=1, …, 3), with $(LC)_{ij}$ the interaction between line and cycle, and e_{ij} the random error.

An additional model was fitted for ESW, again using the GLIMMIX procedure, where the linear regression of ESW on WT was included in the model [1]. The model fitted was:

$$Y_{ijk} = \mu + L_i + C_j + (LC)_{ij} + \beta_i X_{ijk} + e_{ijk} \quad [2]$$

where Y_{ijk} was the ESW of chicken k of antibody line L_i (i=1and 2 for lines LAS and HAS, respectively), from cycle C_j (j=1, …, 3), with $(LC)_{ij}$ the interaction between line and cycle, and X_{ijk} the WT of chicken k from line i at cycle j, and β_i the regression of EST on WT nested within line. In both models, cycle was considered a repeated measure with chicken nested within line as the subject. An auto-regressive covariance structure among periods with homogenous variances was assumed. This structure also assumed equal spacing of sampling events and non-equal correlations across cycles.

Orthogonal contrasts were constructed to test the difference between the antibody lines, the difference between the first and second cycle, and the difference between the average of cycles 1 and 2 and cycle 3. The interactions between lines and cycles were also tested using the two additional orthogonal contrasts. The least squares means for the interactions of lines and cycles were separated using the slice feature in SAS and evaluated using an adjusted Tukey-Kramer test. Correlation coefficients were calculated with the CORR procedure of SAS for pair wise combinations of all egg traits within line.

Mycoplasma gallisepticum. Titers for antibody response to MG were analyzed with the GLIMMIX procedure of SAS fitting the model:

$$Y_{hijk} = \mu + G_h + L_i + S_j + (GL)_{hi} + (GS)_{hj} + (LS)_{ij} + (GLS)_{hij} + e_{hijk} \quad [3]$$

where Y_{hijk} was the titer value from chicken k of sex G_h (h=1 or 2 for hens and roosters, respectively), from antibody line L_i (i=1, …, 3 for lines IC, LAS and HAS, respectively), and sampling period S_j (j=1 or 2), with $(GL)_{hi}$ the interaction of sex and line, $(GS)_{hj}$ the interaction of sex and sampling period, $(LS)_{ij}$ the interaction of line and sampling period, $(GLS)_{hij}$ the three-way interaction of sex, line and sampling period, and e_{ijk} the random error. Sampling period was

considered a repeated measure, with chicken nested within line the subject. An auto-regressive covariance structure among periods with homogenous variances again was assumed, with equal spacing and non-equal correlations between samplings.

Eleven orthogonal contrasts were constructed to test for differences among means. Two contrasts were defined to test for differences among lines. The first contrast tested for difference between antibody lines, while the second contrast tested the difference between the average of the antibody lines and the IC line. Additionally, orthogonal contrasts were constructed to test for differences between the hens and roosters, and between the first and second sampling. Orthogonal contrasts were further constructed to test all two-way interactions, as well as the three-way interaction. The least squares means for all interactions were separated using the slice feature in SAS and evaluated using an adjusted Tukey-Kramer test.

RESULTS

Least squares means for egg shape, weight and quality traits are provided in Tables 3.1 and 3.2.

Egg Shape. The antibody lines differed in overall EW, EL and SI ($P < 0.006$). The HAS line had wider eggs (0.098 ± 0.021 cm) while the LAS line had longer eggs (0.210 ± 0.018 cm), and these differences resulted in the larger SI for the HAS than the LAS line (4.12 ± 0.55). Egg width and EL increased with production cycle, both when comparing cycle 1 to 2 and when comparing the earlier (average of cycle 1 and cycle 2) to the late cycle (cycle 3) ($P < 0.03$). There was no effect of cycle on SI (in cycle 1 vs. 2, $P = 0.06$; in earlier vs. late production, $P = 0.81$).

An interaction between line and cycle was observed for EW both when comparing cycle 1 to 2 and the earlier to the late cycle ($P < 0.001$). When examining EL, there were no interactions among lines and cycles (in cycle 1 vs. 2, $P = 0.76$; in earlier vs. late production, $P = 0.11$). An increase in EW across cycles was more evident in the HAS line, while an increase in EL was more evident in the LAS line. These trends caused a decrease in SI across cycles in the LAS line, and an increase in SI in the HAS line ($P < 0.005$).

Egg Weight. Egg weight increased over production cycles in both antibody lines. However, mean WT did not differ between lines ($P = 0.52$). Average WT increased by 2.51 ± 0.23 g between cycles 1 and 2 ($P < 0.001$). The average WT at cycle 3 was 4.87 ± 0.20 g ($P < 0.001$) heavier than the mean of the earlier cycles. An interaction between antibody lines and cycles for WT was observed when comparing cycles 1 and 2 and when comparing the earlier to the late cycle ($P < 0.001$).

Eggshells. In contrast to WT, ESW was different between the antibody lines with the LAS eggshells weighing approximately 0.67 ± 0.07 g more ($P < 0.001$). Over the course of the three cycles, ESW increased in both lines; the increase in ESW from cycle 1 to cycle 2 was 0.18 ± 0.04 g and the increase from the average of the early cycles to the late cycle was 0.44 ± 0.04 g ($P < 0.001$). An interaction between antibody lines and cycles was observed for ESW when comparing cycles 1 and 2 ($P = 0.01$), but not when comparing the average of the earlier to the late cycle ($P = 0.64$)

Eggshell thickness differed between antibody lines, with thicker shells for the LAS (0.034 ± 0.004 mm) than the HAS line ($P < 0.001$). Across the lines, there was no difference in EST between cycle 1 and cycle 2 ($P = 0.21$). Conversely, there was a difference when

comparing the early cycles to the late cycle, where the increase in EST was about 0.014 ± 0.003 mm ($P < 0.001$). A line by cycle interaction was due to a difference between cycles 1 and 2 ($P = 0.008$), but not between the average of the early cycles and the late cycle ($P = 0.72$). In addition to analysis of EST means, EST variances were also examined. Typically, when means increase, variances increase as well. However, no difference was observed between the variance in the EST measures between the antibody lines ($P = 0.60$), when comparing cycles 1 and 2 ($P = 0.37$), or when comparing the average of the earlier to the late cycle ($P = 0.16$).

Albumen. Albumen height differed between the antibody lines (HAS: 5.60 ± 0.09 mm; LAS: 5.32 ± 0.09 mm; $P = 0.03$) and decreased over the 3 cycles. The AH at cycle 1 was 5.74 ± 0.07 mm, which decreased by 0.32 ± 0.06 mm at cycle 2 ($P < 0.001$). By cycle 3, AH had decreased 0.37 ± 0.06 mm further when compared to the average of cycles 1 and 2 ($P < 0.001$). There were no interactions between lines and cycles for AH ($P > 0.12$).

Haugh units (Haugh, 1937), like AH, were different between antibody lines (LAS: 75.86 ± 0.64; HAS: 77.76 ± 0.64; $P = 0.04$). Similar to AH results, HU also decreased over the course of the three cycles. On average HU at cycle 1 was 80.06 ± 0.55 HU, with a decrease of 3.26 ± 0.51 HU at cycle 2 ($P < 0.001$). By cycle 3, the HU was 4.83 ± 0.51 HU lower as compared to the average of the earlier cycles ($P < 0.001$). As with AH, there was no interaction between lines and cycles for HU ($P > 0.24$).

Yolk Color. As a final indicator of egg quality, YC was assessed. There were no differences in YC between antibody lines ($P = 0.55$) or across cycles ($P > 0.14$), nor was there an interaction ($P > 0.30$).

Egg Quality Correlations. Correlations obtained for all combinations of egg traits within each antibody line are provided in Table 3.3 The strongest relationship within each line was the correlation between AH and HU (LAS: 0.956; HAS: 0.944), both assessments of albumen quality. The second and third strongest correlations for each line were between EW and WT (LAS: 0.840; HAS: 0.914) and between ESW and EST (LAS: 0.818; HAS: 0.891). A strong, positive correlation between ESW and WT in both lines was also observed (LAS: 0.768; HAS: 0.759).

Mycoplasma gallisepticum. Least squares means for antibody titer response to MG are listed in Table 3.4. All main effects of line, sex and sampling period were significant, as were all two-way interactions and the three-way interaction ($P < 0.001$). The MG antibody titer differed between the two antibody lines ($P < 0.001$) with the HAS line having a higher average titer value (160.23 ± 11.14) than the LAS line (48.63 ± 11.64). The average titer value of the two antibody lines was also greater than that of the IC line (53.67 ± 10.25) ($P < 0.001$). Roosters had an average titer of 126.21 ± 10.63, which was more than two and a half times the average response of the hens ($P < 0.001$). Lastly, titer values were higher at the second sampling than the first ($P < 0.001$).

When examining MG antibody response among lines, interactions were observed with sex and sampling event when comparing the HAS and LAS lines ($P < 0.001$) but not when comparing their average to the IC line ($P > 0.06$). These interactions were, in part, because HAS line roosters had titers more than 3 times that of HAS hens, and more than 4 times higher than in LAS line roosters. Additionally, the titer value in the HAS line at the second sampling was more than 5 times higher than at first sampling, and more than 4 times higher than in the LAS line at the second sampling. Additionally, there was an interaction of sex and sampling (*P*

< 0.001) due to the roosters at the second sampling having an average titer value of more than 3 times that of any other group.

Lastly, there was a three-way interaction of line, sex and sampling ($P < 0.001$) due to the very large average MG antibody titer of HAS line roosters at the second sampling. This interaction was not observed when comparing the antibody lines to the IC line ($P = 0.10$).

DISCUSSION

The antibody lines considered in this study have not previously been examined for egg quality traits, with the exception of WT. Thus, comparative information from earlier generations of selection in these lines is unavailable. However, there is a considerable body of literature on egg quality traits useful for placing our results into context. Although some results observed in this study follow similar trends in egg quality traits as reported elsewhere, such was not entirely the case. Therefore, our findings offer new insights into the effects of genetic selection for antibody response to SRBC from a common founder population on egg traits.

Egg Shape. The difference in SI between antibody lines observed in this study is consistent with those reported elsewhere. Van den Brand et al. (2004) found a larger average SI in their high as compared to low antibody line. However, SI values for both their antibody lines were within the range of normal eggs, while in this study both lines had eggs classified as sharp. The differences in SI between these sets of antibody lines may be due to breed differences, as was seen when comparing 32 wk old White Plymouth Rock hens to a cross of Labelle and line "C" hens (Popova-Ralcheva et al., 2009).

Changes in SI over cycles as hens aged were in part consistent with the findings of van den Brand et al. (2004) and Popova-Ralcheva et al. (2009). In the current study, SI increased in

eggs from HAS hens across cycles because EW increased proportionally more than EL as the hens aged. The converse was true in eggs from LAS hens, with a decline in SI. Van den Brand et al. (2004) and Popova-Ralcheva et al. (2009) reported an overall decrease in SI as hens aged. However, in the study by van den Brand et al. (2004), they considered SI in all lines combined. As a consequence, any slight increases in SI in their high antibody line could have been masked by larger decreases in their low antibody and control lines. Since they did not provide SI values over time by line, this is only speculation.

Egg Weight. In the current study WT increased with hen age, which is consistent with previous literature (Silversides, 1994; Ledur et al., 2002; Silversides and Budgell, 2004; van den Brand et al., 2004). As hens age, their eggs typically increase in weight until the first molt, and then WT levels off (Tharrington et al., 1999). There was no difference between the HAS and LAS lines in WT, which was consistent with observations from earlier generations (6 through 8) in these lines (Siegel et al., 1982). Egg weight in the antibody lines of van den Brand et al. (2004), however, did differ in generation 22 of selection where the low antibody line had heavier eggs (1.32 ± 0.25 g) than the high antibody line. The difference in WT distributions in these separate sets of antibody lines may be due to differences in resource partitioning between breeds. The lines considered by van den Brand et al. (2004) were established from ISA Warren medium heavy layers while our antibody lines were established from White Leghorns (Siegel and Gross, 1980). The ISA Warren hens may be sacrificing resources normally used for egg production, specifically egg size, when producing high antibody responses. Conversely, the White Leghorns may have sufficient resources for producing an antibody response without sacrificing egg size.

Eggshells. Eggshell weight increased over the course of the 3 cycles in both antibody lines. Previous literature is ambiguous on trends in ESW as hen age increases. In 6 lines of

hens, Silversides (1994) reported ESW increased between 30 and 45 wk of age, and then decreased between 60 and 75 wk of age. Papova-Ralcheva et al. (2009) reported both increases and decreases in ESW among 6 groups of hens from 32 to 50 wk of age, while Silversides and Budgell (2004) observed no changes in over a 30 wk study considering Brown Leghorn, ISA Brown and Babcock lines of chickens. The increase in ESW observed in the current study can partially be explained by increased WT over time because, when adjusting ESW for WT (model [2]), there was no change in ESW across cycles. Thus, the increase in ESW as hens aged was due to eggs increasing in weight and therefore in physical size.

Eggshell weight, unlike WT, differed between antibody lines, with the eggshells in the LAS line weighing more than those in the HAS line. When adjusting for WT the difference unsurprisingly persisted. Although van den Brand et al. (2004) did not report egg shell weights in their antibody lines, they observed an increased shell percentage in high as compared to low antibody line. Because their high antibody line had lighter egg weights than their low line, direct comparison with our results is difficult.

The increased ESW in the LAS line may indicate LAS hens retain eggs in the shell gland slightly longer than their HAS line counterparts. Increased time in the shell gland would allow more calcium to be deposited, increasing ESW. Hens selected for lower antibody response may also have more resources to devote to the production of the eggshell, resulting in heavier weights compared to the HAS line. Since high antibody production may coincide with a higher demand for protein, it may affect protein transport and calcium channel translocation across the oviduct. The LAS line may have larger protein reserves to support calcification of the eggshell.

The EST also differed between the antibody lines and over time, with eggshells from the LAS line being thicker than those from the HAS line, and increasing over cycles. While the EST means differed, the variance of these measures did not, suggesting that the eggshells increased in thickness equally across the entire shell.

Previous literature suggests that, as hen age increases, EST should remain constant (van den Brand et al., 2004), or even decrease as hens age and eggs become larger (Anderson et al., 2004; Kemps et al., 2006). Van den Brand et al. (2004) found the shells of their high line to be thicker than their low line, while here the opposite was observed. This increased thickness could account for the increased shell percentage in their high line. In the current study, ESW increased in the LAS line. We proposed this may be due to an increase in egg retention time in the shell gland, which would also account for the increased thickness of LAS eggshells.

Albumen. The height of the albumen decreased across cycles in both antibody lines and is consistent with literature that as hens age AH and therefore egg quality decreases (Doyon et al., 1986; Williams, 1992; Silversides, 1994; Ledur et al., 2002; van den Brand et al., 2004). Additionally, AH differed between the HAS and LAS lines, with the HAS line having thicker albumen and therefore superior quality. This result is inconsistent with that of van den Brand et al. (2004), who reported thicker albumen in their low as compared to high antibody line. As anticipated, results for HU were similar to those for AH: the HU measures decreased over time and were superior in the HAS line. Williams (1992) reported that HU scores decrease by about 1.5 to 2.0 units per month while a hen remains in production.

Yolk Color. There was no effect of line, cycle, or their interaction, on YC. Yolk color is predominantly determined by the diet (Galobart et al., 2004) and the diets were the same for both

lines throughout the study. Thus a change in YC was not anticipated, nor observed, as hens aged.

Egg Quality Correlations. A multitude of traits affect egg quality, with correlations among them. In this study, each quality trait measured was correlated with at least one other. Within each line, the correlation between AH and HU was nearly one (LAS: 0.956; HAS: 0.944). These two traits measure essentially the same attribute, albumen height, but in slightly different ways. Thus, it was to be expected that these two measures would be strongly related, as confirmed by Aygun and Yetisir (2010) who reported a correlation of 0.95 ± 0.01 between AH and HU.

The correlations between EW and WT also were extremely high (LAS: 0.840; HAS: 0.914), and somewhat larger than reported recently (0.70 ± 0.01; Aygun and Yetisir, 2010). Aygun and Yetisir (2010) also reported a correlation of 0.60 ± 0.02 between EL and WT, similar to that in the antibody lines (LAS: 0.650; HAS: 0.600).

White Leghorns have been reported to produce eggs with 59.6% albumen contents (Hafez et al., 1954). Albumen weights also increase as hen age increases (O'Sullivan et al., 1991; Silversides, 1994; Silversides and Budgell, 2004). It follows that increasing albumen weights will cause an increase in WT, EL and EW. Increases in albumen weight, however, are not synonymous with increases in AH. Albumen height decreases as hen age increases, which resulted in a negative correlation of -0.38 between WT and HU in both lines.

Shape index was correlated positively with EW (LAS: 0.57; HAS: 0.56) and negatively correlated with EL (LAS: -0.70; HAS: -0.63). Since SI is defined as the EW divided by the EL (multiplied by 100) (van den Brand et al., 2004) the directions of these relationships are as

expected: as EW increases, SI increases, but as EL increases, SI decreases. Recent literature reports a correlation between SI and EW among white egg layers of 0.46 ± 0.03, and a correlation between SI and EL in the same hens of -0.63 ± 0.02 (Aygun and Yetisir, 2010).

Other significant relationships among quality traits were observed. The correlations between ESW and EST were strong (LAS: 0.818; HAS 0.891). For these same traits, Zhang et al. (2005) reported a correlation of 0.45 in a pure line of brown-egg layers, while Kim et al. (2005) reported a correlation of 0.83 in White Leghorns at the end of their second egg production cycle. The strong correlation between ESW and EST is to be expected as thicker eggshells are necessarily heavier. The correlations between ESW and WT in this study were also quite strong (LAS: 0.768; HAS: 0.759). Zhang et al. (2005) reported a correlation of 0.50 in brown-egg layers, while Kim et al. (2005) reported a correlation of 0.32 in White Leghorns at the end of their second production cycle.

Mycoplasma gallisepticum. The HAS line had a higher antibody response to MG than the LAS line (111.6 ± 16.1; $P < 0.001$). This was to be expected, as chickens selected for high antibody response to SRBC may also be more sensitive to other types of immune challenges (Gross et al., 1980; Gross et al., 2002).

There was also a greater antibody response at the second than first sampling (107.0 ± 12.5; $P < 0.001$). The first sampling was conducted after the chickens had been housed in the facility for as long as 16 wk, while the second sampling was conducted after the chickens had been housed in the same facility for more than 32 wk. The increased exposure between the early and late samplings allowed chickens to produce a greater antibody response to the MG challenge.

An unexpected difference in antibody response to MG was evident between roosters and hens. Historically, no sex effects have been evident when examining antibody response to SRBC (Siegel et al., 1982; Kuehn et al., 2006) and we expected that to continue for antibody response to MG. However, in the current study, roosters had a greater response than hens (77.4 ± 12.7; $P < 0.001$). While there was no difference in SRBC antibody response in roosters as compared to hens, the increased response to MG in the roosters instead may have been due to increased exposure time; they were moved into the housing unit 3 wk before the hens. Typically, antibody response to SRBC peaks, and then declines, after a single inoculation; conversely, MG presents a constant challenge.

Conclusions. Overall, egg quality traits were slightly more favorable in the line selected for high than low antibody response. Eggs from hens from the HAS line were shorter and less narrow, resulting in SI values that were closer to normal. Eggs with normal SI scores are more ideal as they conform to pre-made packaging. The greater sharpness of the eggs from the LAS line makes them more susceptible to breakage. While WT and YC did not differ between the antibody lines, the AH and HU measures also were superior in the HAS line. Thicker albumen measures indicate an egg of greater interior quality. In our evaluations of reproductive soundness (Chapter 2), there appeared to be a cost for greater antibody response: commencement of lay was later and fertility was lower in HAS hens. However, at least in terms of albumen attributes, there appeared to be no penalty for selection of high antibody response.

Eggshells produced by LAS hens were heavier and thicker than those from HAS hens. While thicker eggshells are desirable to a certain degree, that advantage in LAS line eggs may be lost because of their sharper shape. Further research is needed to determine if the heavier and thicker eggshells in eggs from LAS hens compensates for their lower SI scores, reducing risk of

breakage. It can be concluded that selection for increased antibody response among White Leghorn hens does not sacrifice egg quality.

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Table 3.1 Least squares means for egg shape traits by line [low antibody select (LAS) and high antibody select (HAS) response lines] across cycle (1 through 3).

Trait	Cycle 1	Cycle 2	Cycle 3	Max. SEM
Egg Width (mm)*				
LAS	3.76[b]	3.78[b]	3.85[a]	0.02
HAS	3.79[c]	3.89[b]	4.02[a]	0.02
Egg Length (mm)§				
LAS	5.69[b]	5.81[ab]	6.03[a]	0.03
HAS	5.53[a]	5.66[a]	5.71[a]	0.03
Shape Index†				
LAS	66.2[a]	65.2[b]	64.0[c]	0.43
HAS	68.6[b]	68.8[b]	70.4[a]	0.43

[abc]Means within a row with different superscripts are different ($P < 0.05$)

* For egg width, lines differed at cycles 2 and 3 ($P < 0.05$)

§ For egg length, lines differed only at cycle 3 ($P < 0.05$)

† Within the trait, lines differed at all three cycles ($P < 0.05$)

Table 3.2 Least squares means for egg weight and quality traits by line [low antibody select (LAS) and high antibody select (HAS) response lines] across cycle (1 through 3).

Egg Trait	Cycle 1	Cycle 2	Cycle 3	Max. SEM
Egg Weight (g)‡				
LAS	45.2[c]	46.7[b]	50.3[a]	0.5
HAS	44.2[c]	47.7[b]	51.4[a]	0.5
Eggshell Weight (g)†				
LAS	4.71[b]	4.76[b]	5.15[a]	0.06
HAS	3.91[c]	4.21[b]	4.52[a]	0.06
Eggshell Thickness (mm)†				
LAS	0.384[ab]	0.379[b]	0.397[a]	0.004
HAS	0.343[b]	0.354[ab]	0.362[a]	0.004
Albumen Height (mm)‡				
LAS	5.66[a]	5.29[b]	5.01[b]	0.10
HAS	5.82[a]	5.56[b]	5.42[b]	0.10
Albumen Height (HU)‡				
LAS	79.3[a]	76.0[b]	72.2[c]	0.8
HAS	80.8[a]	77.6[b]	74.9[c]	0.8

[abc]Means within a row with different superscripts are different ($P < 0.05$)
†Within the trait, lines differed at all three cycles ($P < 0.05$)
‡Within the trait, lines did not differ at any cycle ($P > 0.05$)

Table 3.3 Correlations for all egg trait measurements by line [low antibody select (LAS) and high antibody select (HAS)]. Values above and below the diagonal represent correlations for the HAS and LAS lines, respectively.

	Egg Trait								
	WT	EL	EW	SI	AH	HU	YC	ST	SW
WT		0.60**	0.91**	0.21*	-0.09	-0.38**	-0.05	0.47**	0.76**
EL	0.65**		0.28**	-0.63**	-0.15	-0.30**	0.17	0.22*	0.40**
EW	0.84**	0.18*		0.56**	-0.05	-0.33**	-0.15	0.40**	0.67**
SI	0.07	-0.70**	0.57**		0.09	0.00	-0.27**	0.13	0.19*
AH	-0.10	-0.49**	0.22*	0.57**		0.94**	0.02	0.06	0.04
HU	-0.38**	-0.63**	-0.03	0.51**	0.96**		0.03	-0.10	-0.20*
YC	-0.06	-0.03	-0.04	0.00	0.05	0.06		-0.04	-0.10
ST	0.41**	0.27**	0.30**	0.00	-0.17*	-0.28**	-0.18*		0.89**
SW	0.77**	0.52**	0.60**	0.01	-0.19*	-0.40**	-0.19*	0.82**	

WT = egg wt., EL = egg length, EW = egg width, SI = shape index, AH = albumen height, HU = Haugh units, YC = yolk color, ST = shell thickness, SW = shell weight

** $P < 0.01$

* $P < 0.05$

Table 3.4 Least squares means for antibody titers in response to *Mycoplasma gallisepticum* by sex and sampling, across line [intercross (IC), low antibody select (LAS) and high antibody select (HAS) response lines].

	Line			
Sex	IC	LAS	HAS	Max. SEM
Rooster				
Sampling 1	18.3[b]	37.8[a]	64.2[b]	26.3
Sampling 2	133. 5[a]	78.1[a]	425.5[a]	27.7
Hen				
Sampling 1	11.0[b]	31.5[a]	41.4[b]	18.0
Sampling 2	51.9[ab]	47.2[a]	109.9[b]	18.6

[abc]Means within a column with different superscripts are different ($P < 0.05$)

Chapter 4: Conclusions

Heather N. Albrecht

Reproductive soundness of hens and roosters is important in most commercial poultry operations. The ability of both sexes to efficiently reproduce is central to producing future generations of chickens. With ever changing demands for specific production traits within the industry, selection programs are constantly being developed and revised to meet the desires of the consumer. When specific selection pressures are applied, resources of the chicken must be re-allocated, accordingly. It is necessary that resources needed for increased selection of a specific trait must come from somewhere. Biological processes to which resources typically are allocated include maintenance, growth, reproduction and immunity.

The chickens in this study were progeny of lines selected for high (**HAS**) or low (**LAS**) immune response to SRBC for 36 generations. I concluded that hens from the HAS line commenced lay later and at heavier BW than hens from the LAS line, but with eggs of the same weight. I speculate that the selection for increased antibody response involved the use of resources necessary for hens to commence lay. Hens selected for their low antibody response were able to begin production earlier and at a lighter BW, due to the greater availability of resources for these traits than their HAS line counterparts. Earlier production provides producers the ability to incorporate new hens into a production system sooner. While resources necessary for high antibody response may, in part, have come from the hen's ability to commence lay, they did not appear to come from the production of the egg. The average weight of the first egg was similar for both HAS and LAS hens, suggesting that resources needed for production of eggs were sufficiently available in both lines.

Similarly to the weight of the first egg, the intensity of lay was the same between the HAS and LAS hens for the majority of the periods examined. Consistent lay intensities between lines indicated that neither selection for high nor low antibody response caused a decrease in resources available for the intensity of egg production. These antibody lines also had increased intensity when compared to an unrelated control line for a majority of the periods examined. The decreased intensity in the intercross (**IC**) line may be due to breed differences. Additionally, the difference may indicate that those hens are more susceptible to the ageing process than the antibody lines, suggesting selection for antibody response resulted in a correlated response to increased production intensity.

A major factor in determining reproductive soundness is the length of fertility. Length of fertility was longer in both hens and roosters selected for low antibody response, compared to those selected for a high response. Hens had longer fertility length during cycle 1, while roosters had longer fertility lengths during cycles 1 and 2. Decreased fertility in chickens selected for greater antibody response, again, may indicate that resources needed for the production of antibodies are coming from reproductive resources, specifically those from fertility. Differences were only noted between the lines at the early cycles, suggesting that line differences in the later cycles may be masked by age effects of reduced fertility.

Overall, I can conclude that selection for high antibody response in chickens caused a decrease in generalized reproductive soundness. In this study only commencement of lay parameters and length of fertility were analyzed, while other reproductive factors including percent of early and late dead embryos, as well as hatchability factors, were not examined. Furthermore, a control line of White Leghorns, not selected for antibody response, would have better facilitated comparisons of commencement of lay and fertility length among the antibody

lines. That is, an unselected line of the same breed raised under the same conditions, would have provided an ideal baseline for comparison. Further research is warranted to fully evaluate reproduction to determine if selection for high antibody response has negative effects in all areas. Results from this study imply that the resources needed to produce an increased antibody response may have come from resources that would have otherwise gone towards reproduction. This trend, of sacrificing reproductive resources, may follow in other selection experiments, not just those for immunocompetence.

Differences existed between the antibody lines in regards to egg traits, though the differences were not in the direction initially expected. It was hypothesized that the LAS line would have more favorable egg traits than their HAS counterparts, but such was not the case. The hens from the HAS line had slightly more favorable egg traits overall, including shorter and wider egg dimensions, resulting in more normal shape index (**SI**) scores, and greater albumen height (**AH**) and Haugh unit (**HU**) measures. The LAS hens did have heavier and thicker eggshells, compared to the HAS hens, possibly indicating stronger shells, while both egg weight and yolk color did not differ between lines.

Heavier and thicker eggshells in eggs from LAS hens were the only quality traits found to be superior in that line. While these traits may indicate stronger shells, eggshell strength was not measured in this study. The more extreme shape of these eggs may result in difficulty in packaging, increasing the incidence of breakage, regardless of the weight and thickness of the shells.

It was suspected that the LAS hens would have more favorable egg trait measures due to more available resources to invest into production, compared to hens selected for high antibody

response. Egg weight did not differ between the lines, indicating that the weight of the eggs was not influenced by selection for antibody response. However, AH, HU and SI were superior in the HAS hens indicating that resources needed to produce eggs with these more favorable qualities were not reduced due to the selection. The superiority, compared to the LAS line, may even indicate that selection for high antibody response has a correlated response of improvement in certain egg traits.

Similar to the fertility study, the use of a control line of White Leghorns, not selected for antibody response, would have facilitated comparisons of egg trait measures among the antibody lines. A line raised under the same conditions, but with no prior antibody selection, would have provided a baseline for egg trait measures to be compared. This would help determine if the differences observed in the antibody lines were collectively better or worse than in an unselected control line of the same breed, but to different degrees, or if high and low antibody selection resulted in traits that were superior to controls in one line yet inferior in the other.

Antibody response to *Mycoplasma gallisepticum* (**MG**) was suspected to differ between the antibody lines. As expected, the HAS line expressed a greater response to MG. Surprising, however, were the differences observed between roosters and hens. We suspected no difference between sexes, as no difference was evident in their response to SRBC. Roosters, however, produced a greater antibody response to MG, possibly due to increased exposure to the MG infection than hens.

Final Conclusions

Additional research facilitating a comparison of the selection lines to an unselected control line of the same breed would provide more comprehensive results of reproductive

soundness and egg quality, as a result of the selection. This type of project would give a better understanding of the correlated responses in these areas due to the selection for antibody response.

Selection programs, of all types, have the potential to create undesirable outcomes in production traits not associated with the specified selection. In this study, we considered the impact of selection for antibody response to SRBC on reproductive soundness, egg quality and resistance to MG. While there was a negative impact on reproductive soundness, such costs were not evident in egg quality. Correlated responses in unselected traits are not always undesirable, and should not deter the creation of other selection programs.