SAFETY AND QUALITY OF NON-COMMERCIAL SHELL EGGS

by

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Dedication

To my four extraordinary children, Aaron II, Jack, Taylor and Luke. Throughout the program at Auburn University, I may have missed significant events in your lives. At the time, I was riddled with guilt, as these events seemed so immensely important. In hindsight, I realize they were character building moments for me *and* for all of you. I am grateful that through it all, each of you have grown and strengthened in more ways than I ever could have imagined. I am proud to look into your eyes and echo a valuable lesson to work effectively, delay gratification, and achieve goals. Lastly, I am humbled and hopeful to be your example of always finishing what you start. With all my love - MOM

Abstract

The market for non-commercially sourced shell eggs has primarily been driven by consumers' perception of higher safety and quality. Due to the marginal availability of data on these eggs, the objective of this study was to evaluate the safety and quality of non-commercial shell eggs to provide data to its current state, as well as establish a baseline for reference. Convenience sampling of shell eggs were derived from small/backyard flocks, small farms, community farms, farmers markets, and/or health food stores. Safety evaluation focused on microbiological (specifically, *Salmonella* spp.) and chemical (specifically, lead and arsenic) as a potential hazard. For detection of *Salmonella* spp. (n=1,388) (isolated by FDA BAM culture methods and confirmed through PCR), 3 positive samples were identified, which suggests that the risk of *Salmonella* is higher in shell eggs from non-commercial sources compared to those produced by commercially caged hens (1 in 20,000). In a rural backyard flock case study, composite eggs (yolk and albumen) analysis (n=36)(ICP-MS) indicated lead and arsenic concentration ranges from <0.02 ppm to 0.05ppm and <0.02 ppm to 0.04 ppm, respectively, where environmental soil analysis (ICP-AES) indicated lead and arsenic detection at 1,835 ppm and 6 ppm, respectively. The presence of lead and arsenic from this case study, suggests a potential public health concern from non-commercial shell eggs. With quality evaluation, yolk color data (n=1,118) was conducted with a novel digital yolk colorimeter (Digital YolkFan™) and indicated an average of 9.0. Haugh unit (HU) data (n=1,273) indicated an average of 75.24 HU. Based on collected HU values and equivalence to USDA grading, approximately 65% of all samples evaluated were Grade AA, 25% were Grade A, and 10% were Grade B or less. Average shell strength (n=1,273) was observed at 3,939 g Force, which is within the average range of commercially produced brown and white eggs. Average vitelline membrane strength (n=1,191)

was observed at 122.79 g force, which is below previously evaluated commercial eggs that underwent extended storage. Overall, the quality of shell eggs from non-commercial sources can vary from comparable to retail markets to well below. Finally, non-commercial shell egg suppliers are varied in their flock management and egg handling practices (egg collection frequencies, egg washing, and storage/transport conditions). These variances can contribute to the quality and safety of shell eggs, and is important to consider as a consumer to understand the potential impact to the shell eggs being purchased/obtained.

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LIST OF ABBREVIATIONS

bp	Base pairs
BS	Bismuth sulfite agar
ºC	Degrees Celsius
CDC	Center for Disease Control and Prevention
CSA	Community supported agriculture
HE	Hektoen enteric agar
ICP-MS	Inductively coupled plasma-mass spectrometry
ICP-AES	Inductively coupled plasma-atomic emission spectroscopy
kb	Kilobase pairs
µL	Microliter
mL	Milliliter
PCR	Polymerase chain reaction
RV	Rappaport-Vassiliadis broth
SE	*Salmonella* Enteritidis
TT	Tetrathionate broth
TSB	Tryptic soy broth
U.S.	United States of America
XLD	Xylose lysine desoxycholate agar

CHAPTER I: INTRODUCTION

1.1 Overview of Research

Distribution channels of shell eggs from non-commercial sources (e.g. small and backyard flocks) have expanded over time. Sales have gone beyond personal contacts and roadside stands to include: farmer's markets, health food markets, and farms involved in community-supported agriculture (CSA) programs. Consumer interest in non-commercialized shell eggs are attributed to consumer perceptions of higher quality and safety, relative to their commercially sourced counterparts. Furthermore, the local foods movement also contributes to the rise in consumer demand. The influx in small and backyard flocks has prompted extension specialists to develop and distribute education materials on safe handling practices. Education through extension assist to mitigate potential spread of disease and pathogens associated with managing flocks.

Growth in distribution channels, combined with potential safety risks, increase the likelihood of public health concerns. Potential safety concerns focus on microbiological (e.g. *Salmonella*) and chemical sources (e.g. lead and arsenic). Since data on the safety of shell eggs from small and backyard flocks is limited, further research is necessary to fully understand the extent of probable concern. In addition, data on quality assessment of shell eggs from small and backyard flocks can also provide valuable information to validate/nullify consumer perceptions. Although consumers perceive non-commercial shell eggs with an increased in safety and quality, variable handling, storage, and transportation conditions can contribute to the degradation of the shells eggs prior to reaching the consumer. Thus, the objective of this study is to evaluate the safety and quality of non-commercial shell eggs to provide data to its current state, as well as a baseline for reference.

1.2 Organization of Document

This research begins with background information on consumer interest in non-commercial shell eggs. Subsequent chapters comprise of compartmentalized studies including: *Salmonella* in non-commercial shell eggs; a case study: lead and arsenic contamination in non-commercially sourced shell eggs; egg quality of non-commercial shell eggs; and an evaluation of small flock egg handling. The overall objective of this dissertation is to provide collective research data, and a baseline, on the safety and quality of non-commercial shell eggs.

CHAPTER II: INTEREST IN NON-COMMERCIAL SOURCES OF SHELL EGGS

The popularity of consumer interest in non-commercialized shell eggs is ongoing. Although rural environments tend to be the primary location non-commercialized shell eggs (from small and backyard flocks), live poultry are seen more and more in suburban, as well as urban areas. A survey, which focused on Los Angeles, Denver, Miami, New York City, found that 1% of households owned chickens (Bahravesh and others, 2014). In addition, 4% planned to become first-time chicken owners within 5 years. In another survey conducted nationwide in the United States (Elkhoraibi and others, 2014), respondents thought that eggs/meat from their chickens tasted better (95%), were safer to consume (84%), and were more nutritious (86%) than store-bought products. In addition, they perceived that the health and welfare of their chickens was better (95%) than on commercial farms. These perceptions have contributed to the increase in the poultry mail-order industry.

In 2009, the mail-order industry reported record sales due to increased interest in raising backyard flocks. In the United States, mail-order hatcheries provide an annual estimation of 50 million live poultry sales to the public (Gaffga and others 2012). The U.S. Postal Service delivered from hatcheries to private homes and/or agricultural feed stores around the country. Distribution can travel across state boundaries, be spread through sales in feed stores, or be distributed through other mail-order hatcheries (Gaffga and others 2012).

This national distribution of birds through the mail-order system poses a potential for widespread dispersal of *Salmonella* contamination from a single hatchery. Public health officials have seen *Salmonella* outbreaks linked with poultry sales for backyard flocks and the first multistate outbreak where the backyard flock trend was recognized by the CDC occurred in 2007 (CDC, 2009; Basler and others, 2016). More recently, eight multistate outbreaks of *Salmonella*

infections, covering 45 states, were linked to backyard flocks which included 611 infected individuals, 138 hospitalizations, and one death (CDC 2016). Not only is *Salmonella* of public concern, small and backyard flocks have also been implicated in the potential spread of avian influenza, as the US reported 21 backyard flocks being affected with H5 highly pathogenic avian influenza (HPAI) during 2014-2015 (USDA, 2016).

CHAPTER III: *SALMONELLA* IN SHELL EGGS FROM NON-COMMERCIAL SOURCES

3.1 Abstract

Salmonellosis is the leading cause of foodborne illness in shell eggs, and has been the focus of safety in commercial production. Consumers of non-commercial (e.g. local farms, farmer's markets, health food stores, backyard flocks) shell eggs are motivated by perceptions of higher safety and quality; however, few studies have evaluated the presence of *Salmonella* from these suppliers. The purpose of this study was to evaluate the occurrence of *Salmonella* from non-commercial shell eggs, to further understand the potential for public concern. Convenience random sampling of 1,388 shell eggs were obtained from non-commercial sources in the state of Alabama. Isolation for *Salmonella* was performed according to FDA Bacterial Analytical Manual, modified with elimination of composite sampling. Presumptive positive samples were confirmed by colony polymerase chain reaction (PCR), with *Salmonella* detection primers TS-11 (5'-GTCACGGAAGAAGAGAAATCCGTACG) and TS-5 (5'-GGGAGTCCAGGTTGACGGAAAATTT), and further verified with primers InvA5 (5'-GTGAAATTATCGCCACGTTCGGGCAA) and InvA3 (5'-TCATCGCACCGTCAAAGGAACC). Amplicons were observed through 1.2% agarose gel electrophoresis. PCR results indicated the presence of 3 positive samples for *Salmonella* in the 1,388 evaluated samples. According to this study, the occurrence of *Salmonella* is higher in shell eggs from non-commercial sources, compared to the national average of those produced by commercially caged hens (1 in 20,000). The data suggest that the risk of *Salmonella* is higher in shell eggs from non-commercial sources compared to those produced by commercially caged hens, implying the necessity for additional education and promotion of egg safety and handling

guidelines. However, further nationwide, or compiled state-by-state, evaluations of these non-commercial shell egg suppliers would provide additional insight on the risk to public health.

3.2 Introduction

Consumer interest in shell eggs from non-commercial sources (e.g. small and backyard flocks, local farms, farmer's markets) continues nationwide; however, few studies have evaluated the presence of *Salmonella* from these sources. Salmonellosis is the leading cause of foodborne illness in shell eggs, and *Salmonella Enteritidis* has been the focus of safety in commercial production. Although SE is the most frequently isolated *Salmonella* serotype in shell eggs, it is significant to recognize the impact of other serotypes (e.g. Heidelberg and Typhimurium) and realize a wide evaluation of *Salmonella* occurrence. The purpose of this study was to evaluate the occurrence of *Salmonella* from non-commercially sourced shell eggs, to further understand the potential for public concern.

3.3 Review of Literature

Salmonella Enteriditis (SE) is the most frequently isolated *Salmonella* serotype from layer flocks (Braden, 2006). Frequently, birds infected with SE appear healthy, while passing these pathogens to their shell eggs in two ways. As cited by Gantois and others (2009), there are two possible routes of shell egg contamination by SE. One possible route, considered as horizontal transmission, is when shell eggs contaminated by penetration through the eggshell from the colonized gut or from contaminated feces during or after oviposition. The second possible route, considered as vertical transmission, is by direct contamination of the albumen, yolk, eggshell membranes or eggshells before oviposition. Vertical transmission originates from

the infection of reproductive organs with SE. There appears to be much debate over which route is more significant (Gantois and others, 2009).

According to the FDA, consumption of eggs contaminated with SE causes approximately 142,000 annual illnesses (FDA, 2009). Since only a portion of foodborne infections are diagnosed and reported, many more infections likely occur. Most SE infections result in symptoms such as: abdominal cramps, diarrhea, fever, headache, nausea, and vomiting. In those with compromised immune systems (e.g. the very young, the elderly, and those with immune deficiencies), SE can be very serious and can lead to death (FDA 2009).

In an effort to reduce the incidences associated with SE contaminated eggs, the FDA issued a final Egg Rule (FDA, 2009). The egg rule requires commercial egg producers to take specific preventive measures to keep eggs safe during their production, storage and transport. Egg producers are required to register with FDA and to maintain a prevention plan and records to show they are following the regulation. Furthermore, shell egg producers are required to implement measures to prevent SE from contaminating eggs on the farm and from further growth during storage and transportation (FDA, 2009). On September 8, 2009, the egg rule required compliance by July 9, 2010 for producers with 50,000 or more laying hens. For producers with fewer than 50,000 but at least 3,000 laying hens, compliance was afforded to July 9, 2012. Much of the research on shell eggs have focused on detection and techniques to mitigate SE from a commercial standpoint such as evaluation of housing systems (Gast and others, 2017) and pasteurizing techniques (Caudill and others, 2010). Producers with fewer than 3,000 laying hens and those that sell all of their eggs directly to consumers are exempt from the egg rule (FDA 2009). This exemption allows for wide variances in production, handling, storage, and transportation in small and backyard flocks, which are still susceptible to similar microbiological

safety concerns as their large-scale counterparts.

Although SE is the most frequently isolated *Salmonella* serotype from layer flocks (Braden 2006), other *Salmonella* serovars have also been linked to egg-transmitted illness. For example, contaminated eggs have repeatedly been implicated as the food vehicles for *Salmonella* Typhimurium infections in Australia (Stephens and others, 2008; Slinko and others, 2009). In Tasmania, between 2005 and 2008, 7 egg-related outbreaks of *Salmonella* Typhimurium lead to 191 confirmed cases (Stephens and others, 2008). Slinko and others (2009) confirmed a single egg producer as the source of *Salmonella* Typhimurium which led to foodborne illness outbreaks in 5 separate restaurants in Brisbane. *Salmonella* Heidelberg infections have also been implicated in egg-related foodborne illnesses (Kaldhone and others, 2016; Hennessey and others, 2004; and Chittick and others, 2006). During 1973-2001, Chittick and others (2006) noted 28 foodborne egg-related outbreaks due to *Salmonella* Heidelberg. Hennessey and others (2006) noted approximately 37% of *S*. Heidelberg infections originated from egg-related foods prepared from outside the home.

Although *Salmonella* serovars Enteritidis, Heidelberg, and Typhimurium have been implicated in egg-related foodborne illnesses, it is noteworthy to briefly mention their varied virulence properties. For example, Gast and others (2011) observed both *S*. Enteritidis and *S.* Heidelberg colonized the ovaries and oviducts of infected hens at similar frequencies; however, the occurrence of egg contamination by *S.* Enteritidis (3.58%) was significantly higher relative to contamination by *S*. Heidelberg (0.47%). Gantois and others (2008) observed *Salmonella* serotypes Enteritidis and Typhimurium strains colonized the reproductive organs better than the *Salmonella* strains from serotypes Heidelberg. In the same study, low numbers of *Salmonella* serotypes Enteritidis, Typhimurium, and Heidelberg strains were observed to survive in the egg

albumen during egg formation (Gantois and others, 2008). Virulence studies of shell egg contamination by different *Salmonella* serotypes are extensive, and further focused in other research.

Salmonella contamination in eggs typically contain very low bacterial cell concentrations; therefore, large numbers of eggs must be sampled to achieve a high probability of detection (Gast, 1993a,b,c). In commercial shell egg manufacturing, where there is high volume, pooling is necessary to prevent overwhelming laboratory facilities and resources; however, this pooling process further dilutes the already low *Salmonella* concentrations present in contaminated individual eggs. To encourage multiplication of salmonellae, egg pools are sometimes incubated after mixing (overnight at 37°C or for several days at room temperature) in order to be promote detectable levels before continuing with subsequent bacteriological enrichment culture steps (Gast, 1993a,b,c; Gast and Holt, 2003) .

Detection of *Salmonella* in shell eggs has involved culture methods, molecular methods, and a combination of both. Culture methods, using selective and differential plating media, are simple and a wide variety of media has been developed for this purpose, including: xylose lysine desoxycholate agar (XLD), Hektoen enteric (HE) agar, and bismuth sulfite (BS) agar. XLD and HE agar are the most popular media for isolating *Salmonella* spp., and their differentiation abilities are dependent on characteristics of *Salmonella*, such as hydrogen sulfide production and the non-fermentation of lactose. Unfortunately, these characteristics are shared with *Proteus* spp. and *Citrobacter* spp. As a result, numerous false-positive results are observed on these media (Gaillot and others, 1999; Park and others, 2012). BS agar is the medium of choice for the isolation of *Salmonella* Typhimurium, as well as atypical *Salmonella* that ferment lactose; however, disadvantages include low sensitivity and long incubation time for development of the

characteristic colony morphology (Park and others, 2012; Jay and Davey, 1989). Due to limitations associated with the use of these selective and differential media, further confirmation testing is required, which is a time-consuming and labor-intensive activity (Gaillot and others, 1999; Park and others, 2012).

Polymerase chain reaction (PCR) has been utilized as a time-saving, labor reducing, and highly accurate molecular method of detection. With PCR, DNA is easily and rapidly amplified from pure cultures. However, potential problems occur if the sample investigated is a complex matrix (e.g. clinical specimens or foods, such as shell eggs), since PCR is easily inhibited by many substances, including: humic acids, fats, and proteins. Consequently, selective and differential media has been utilized to isolate *Salmonella*, followed with confirmation by PCR (Jawad and Al-Charrakh, 2016). The effectiveness of PCR is highly reliant on the specificity of primers utilized, and several specific primers have been demonstrated as suitable PCR targets in various food matrices for detection of *Salmonella*. Among them, TS11 with TS5 (Tsen and others, 1994; Kawasaki and others, 2005) and InvA 5 with InvA 3 (Bantacor and others, 2005) have been utilized for detection of *Salmonella* in beef, poultry and eggs. Table 3.1 illustrates sequences, amplicon, and established annealing temperatures of Salmonella primers TS11 and TS5 (Tsen and others, 1994; Kawasaki and others, 2005), as well as InvA5 and InvA3 (Betancor and others, 2010). This study utilized a combination of PCR molecular techniques combined with culture methods, to detect *Salmonella* in shell eggs from small flocks.

Table 3.1 Sequences, amplicon, annealing temperatures, and referenced *Salmonella* primers

Primers	Sequences (5'-3')	Amplicon, annealing temp & source
TS-11	GTCACGGAAGAAGAGAAATCCGTACG	375bp, 59°C, *Salmonella* specific Chromosomal Fragment of HindIII (Tsen and others 1994)
TS-5	GGGAGTCCAGGTTGACGGAAAATTT	
InvA5	GTGAAATTATCGCCACGTTCGGGCAA	285bp, 59°C, *Salmonella* Invasion protein (Betancor and others 2010)
InvA3	TCATCGCACCGTCAAAGGAACC	

3.4 Materials and Methods

3.4.1 Sampling

Convenience random sampling of shell eggs (n=1,273) from 52 small flocks, throughout the state of Alabama (Figure 3.1). Sampling sources included: backyard flocks; health food stores, small farms, and farmers markets. On day of obtaining samples, shell eggs were transported in coolers with frozen gel packs and stored at 7±2 ℃ for 48 h prior to *Salmonella* evaluation.

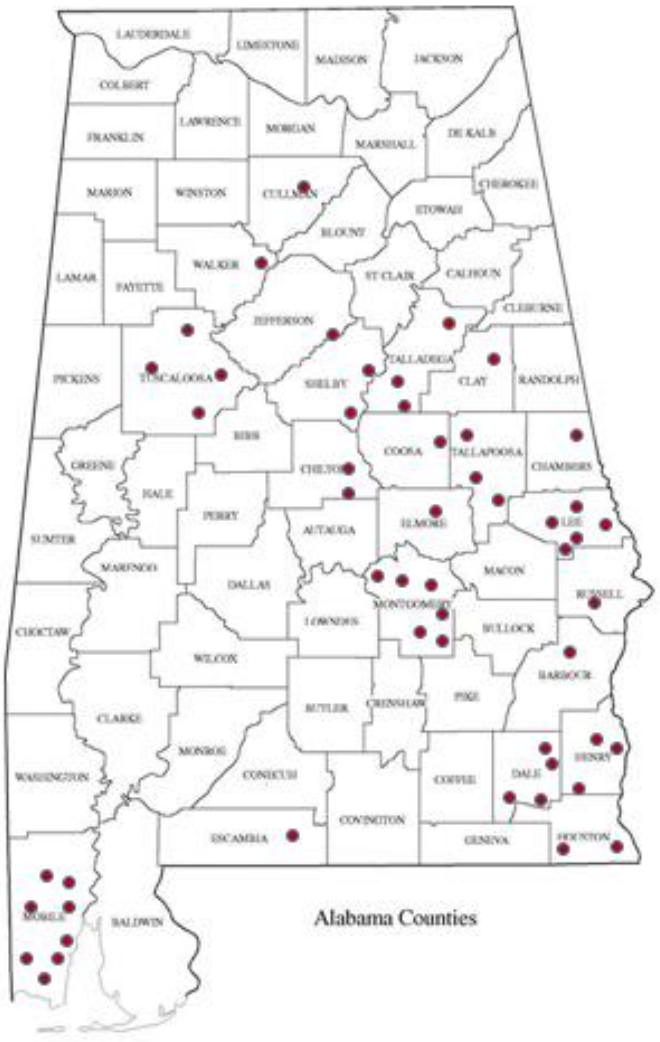

Figure 3.1: Non-commercial shell egg sampling sites for *Salmonella* evaluation (n=52)

3.4.2 *Salmonella* Isolation

Isolation of *Salmonella* was conducted according to FDA Bacterial Analysis of Microbiology (BAM) (FDA, 2017), modified with elimination of composite sampling. In this study, the units of obtainable shell eggs were a limiting factor; therefore, sampling of each individual shell egg enabled a complete assessment of all samples collected. Upon removal from storage, shell eggs were inspected, and cracked samples were discarded/eliminated. Surface of egg shells were disinfected with a 3:1 (vol/vol) solution of 70% ethyl alcohol and iodine/potassium iodide solution, respectively. Iodine/potassium iodide solution consisted of 50 g iodine and 100 g potassium iodide per liter of distilled water. Eggs were submerged in the ethyl alcohol and iodine/potassium iodine disinfectant solution for 10 s. Eggs removed from the disinfectant were air dried, prior to being cracked aseptically into 200 mL sterile sample containers (VWR, Suwannee, GA). Egg samples (yolk and albumen) were thoroughly mixed and incubated at room temperature for 96 ± 2 h. After the 96 h incubation, samples were pre-enriched with 100 mL sterile Tryptic Soy Broth (TSB), and further mixed. Positive control samples (cocktail mix of *Salmonella* Enteritidis, Typhimurium, and Heidelberg) were incorporated. Then, samples were incubated for 24 ± 2 h at 35 °C. Initial pre-enrichment and incubation were followed by secondary enrichment in selective media, by the addition of 1 mL of the egg mixture (pre-enriched culture) to 10 mL Tetrathionate (TT) broth, and another 0.1 mL of the egg mixture to 10 mL Rappaport-Vassiliadis (RV) medium. RV medium was prepared with individual ingredients. Samples in RV medium were held for 24 ± 2 h at 42 ± 0.2 °C, in a circulating, thermostatically-controlled, water bath. Samples in TT broth were incubated for 24 ± 2 h at 35 ± 0.2 °C. After secondary enrichment, RV medium enriched samples were vortexed, and streaked on bismuth sulfite (BS) (Becton, Dickson, and

Company, Sparks, MD), xylose lysine desoxycholate (XLD) (Becton, Dickson, and Company, Sparks, MD), and Hektoen Enteric (HE) agar plates (Neogen, Lansing, MI). BS and HE plates were prepared the day before use, and were stored in a dark, ambient temperature environment until streaked. TT broth enriched samples were similarly streaked in XLD, HE, and BS agar. All plates were incubated for 24 ± 2 h at 35 °C. Presumptive positive samples were evaluated with colony PCR.

3.4.3 Colony PCR

Salmonella Typhimurium (CT18), *Salmonella* Enteritidis (SAB17), *Salmonella* Heidelberg (SARA36) (*Salmonella* Genetic Stock Centre - University of Calgary, Calgary, Canada) served as positive controls; whereas *E. coli* (Top10, Invitrogen, Carlsbad, CA), *Listeria monocytogenes* (ATCC 19111) and *Staphylococcus aureus* (ATCC 12600) were utilized as negative controls. These bacteria were used in a gradient PCR to obtained best annealing temperature and to evaluate the sensitivity and specificity of primers (TS and InvA). Single colonies were picked and suspended in 20 µL PCR certified DNAse and RNAse free sterile water (W3440, Teknova, Hollister, Ca). These suspensions were then stored under refrigeration and served as the DNA template for the PCR reactions. Each PCR sampling was performed with a total volume of 50 µL, which consisted of 1 µL of DNA template and 49 µL of PCR master mix. PCR master mix composed of: 1 µL dNTP (C01581-10 GenScript, Piscataway, NJ); 5 µL 10X EconoTaq reaction buffer with Mg (30031, Lucigen Corp. Middleton, WI); 0.5 µL of each *Salmonella* detection primers TS-11 and TS-5 (Integrated DNA Technologies, Coralville, IA); 0.5 µL EconoTaq polymerase (Lucigen Corp.); and 41.5 µL DNAase and RNAase

free sterile water. PCR were performed with a thermal cycler (iCycler Bio-Rad, Hercules, Ca), and parameters included: an initial denaturation at 95 °C for 2 min followed by 40 cycles of denaturation (95 °C, 30 s), annealing (59 °C, 30 s), and extension (72 °C, 30 s). After a 5 min final extension at 72 °C, PCR products were subjected in agarose gel for electrophoresis. Additionally, *Salmonella* detection primers (InvA5 and InvA3) (Integrated DNA Technologies) were utilized to further confirm with a similar PCR master mix composition and cycle parameters.

3.4.4. Gel Electrophoresis

Ten µL of PCR products were loaded in the wells of a 1.2% agarose gel, in 1X TAE buffer (40mM Tris, 20mM Acetate and 1mM EDTA, pH 8.6), with 0.3 µg/mL ethidium bromide. Through electrophoresis at 5 volt/cm for 60 min, amplicons were observed under UV 302 nm irradiation (C200 Azure Biosystems, Dublin, CA). A 1kb DNA ladder (SM1331, Thermo Fisher Scientific) served as a molecular weight standard, with expected observations of amplicons for *Salmonella* at 375 bp (for TS11 and TS5 primers) and 285 bp (for InvA5 and Inv3 primers).

3.4 Results and Discussion

Of 1,388 samples, a total of 3 shell egg samples were confirmed positive. Table 3.2 represents results of the XLD, HE, and BS culture plates for *Salmonella* with enrichment by RV or TT. Presumptive positive plates were confirmed with colony PCR, which indicated false-positives within the following culture plates: RV/XLD (26), TT/XLD (28), RV/HE (25), TT/HE (29), RV/BS (31), and TT/BS (34). Specificity calculation of TT/XLD (98.0%) was greater than

the specificity of RV/XLD (86.3%), RV/HE (86.1%), TT/HE (86.4%), RV/BS (92.0), and TT/BS (92.4%). Park and others (2012) indicated a lower specificity of RV/XLD (73%), upon detection of *Salmonella* in ground chicken, beef, and ground pork. Differences in specificity between studies can be attributed to the varied food models evaluated, and/or varied supplier of testing materials, and/or varied parameters of confirmation evaluation.

Table 3.2 Specificity of XLD, HE, and BS medium enriched with RV or TT in detection of *Salmonella* spp. in non-commercial shell eggs

Enrichment/Medium	No. of True-Negative Results	No. False-Positive Results	% Specificity [a]
RV/XLD	1359	26	98.1
TT/XLD	1357	28	98.0
RV/HE	1360	25	98.2
TT/HE	1356	29	98.1
RV/BS	1354	31	97.8
TT/BS	1351	34	97.5

[a] (No. of true-negative results on the medium/no. of negative samples [b]) X 100

[b] No. of negative samples = 1385

RV=Rappaport-Vassiliadis broth; XLD=Xylose Lysine Desoxycholate agar; TT=Tetrathionate broth; HE=Hektoen Enteric agar; BS=Bismuth Sulfite agar

PCR confirmation of presumptive positive samples, for *Salmonella*, is illustrated in Figure 3.2. Figure 3.2 shows the agarose gel electrophoresis image of the PCR *Salmonella* amplicons where: Lane M, DNA marker ladder; Lane 1, negative control (*E. coli)*; Lane 2, negative control (*L. monocytogenes*); Lane 3, negative control (*S. aureus*); Lane 4, positive control (S. Enteritidis), Lane 5, positive control (S. Typhimurium); Lane 6, positive control (S. Heidelberg); Lane 7-9, positive samples. According to this study, the occurrence of *Salmonella* in non-commercial shell eggs is 3 in 1388 samples. The results of this study indicate a higher incidence of *Salmonella* in shell eggs from non-commercial sources, relative to the occurrence in shell eggs produced from commercially caged hens, of approximately 1 in 20,000 (Ebel and Schlosser, 2000).

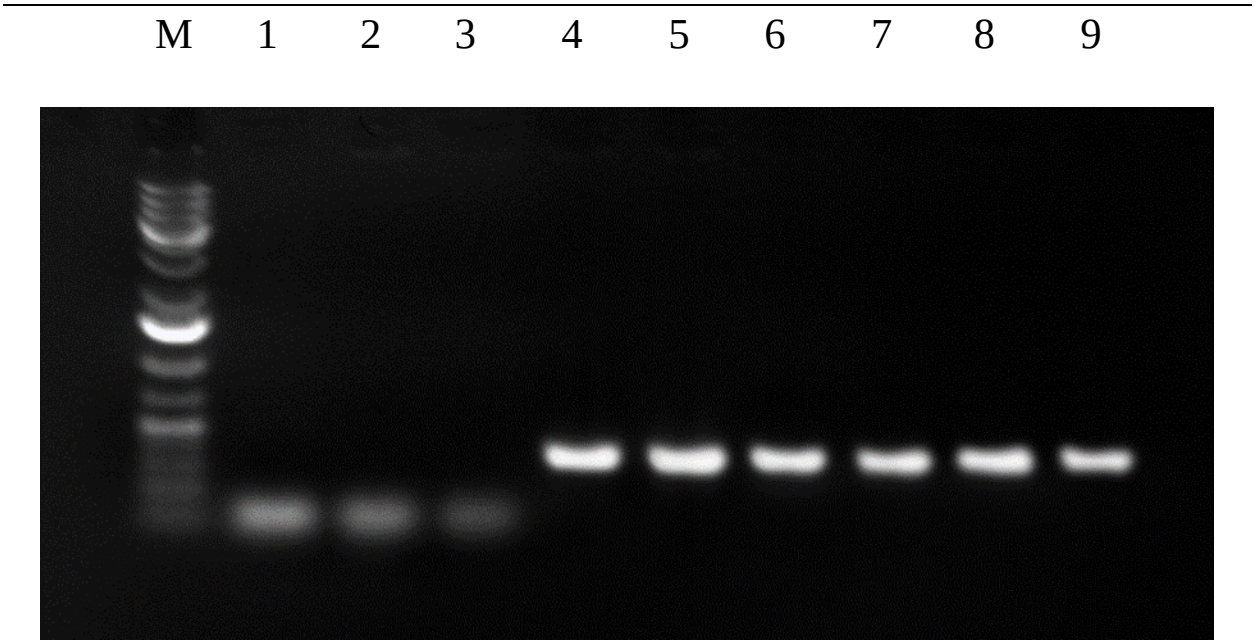

Figure 3.2 Agarose gel electrophoresis image of PCR *Salmonella* amplicons utilizing TS11 and TS5 primers. Lane M, marker DNA ladder; Lane 1, negative control (*E. coli)*; Lane 2, negative control (*L. monocytogenes*); Lane 3, negative control (*S. aureus*); Lane 4, positive control (S. Enteritidis), Lane 5, positive control (S. Typhimurium); Lane 6, positive control (S. Heidelberg); Lane 7-9, positive samples.

3.6 Conclusions

The data suggest that the risk of *Salmonella* is higher in shell eggs from non-commercial sources compared to those produced by commercially caged hens. This implies the potential need for additional egg safety and handling guidelines education/promotion. Although an expansion of population sampling (e.g. nationwide, or compiled state-by-state) of non-commercial shell egg suppliers may provide additional insight on the risk to public health, the current data provides an important baseline and possible trend of what can be expected.

CHAPTER IV: CASE STUDY: LEAD AND ARSENIC EXPOSURE FROM NON-COMMERCIAL EGG SOURCE

4.1 Abstract

Consumer preferences for local (non-commercial) shell eggs are a growing market. Non-commercial sources of eggs, including small local farmers and backyard flocks, vary in flock management and egg handling practices which can negatively affect the quality and safety (biological/chemical) of eggs. In this case study, lead (Pb) and arsenic (As) levels were evaluated in shell eggs from a small backyard flock located in rural northern Alabama. As a potential contaminant source, the flock's environmental soil was analyzed (via ICP-AES), which indicated concentrations of Pb at 1,835 ppm and As at 6 ppm. In perspective, the United States (US) Environmental Protective Agency (EPA) requires clean-up in soils with Pb and As at 400 ppm and 0.4 ppm, respectively. Egg (yolk and albumen) analysis (via ICP-MS) indicated Pb and As concentration ranges from <0.02 ppm to 0.05ppm and <0.02 ppm to 0.04ppm, respectively. Comparatively, the EPA permissible level for As is 0.010 ppm in drinking water; whereas, the Food and Drug Administration states a maximum Pb level of 0.05 ppm in juices and 0.10 ppm in candy. The presence of Pb and As in eggs from non-commercial sources has potential public health risks, which necessitates further investigation.

4.2 Introduction

Distribution channels of non-commercial sources of shell eggs (e.g. small and backyard flocks) have risen over time. Currently, point of sales include: personal contacts, farmer's markets, health food markets, and farms involved in community-supported agriculture (CSA) programs. The surge of general interest for shell eggs from non-commercial sources has been attributed to consumer perceptions of higher quality and safety. In addition, the local foods

movement has continued to influence consumer purchases. Over the years, the rise in small and backyard flocks have prompted extension specialists to develop educational materials on safe handling practices to mitigate potential spread of disease and pathogens associated with managing flocks; however, minimal attention has been given to chemical safety risks in consuming shell eggs from these suppliers.

Literature on chemical safety in shells eggs has identified lead (Pb) and arsenic (As) as potential concerns. During 2010–2012, thirteen confirmed cases (from eleven backyard flocks) of lead intoxication were documented from fifteen different counties in California. Evaluation of these cases indicated subclinical lead toxicity, which made it difficult to identify exposed flocks. In addition, it is suspected that lead poisoning frequencies are underestimated in backyard poultry, due to lack of routine diagnostic procedures in sick or deceased birds. Similarly, this is the case for arsenic intoxication assessment. Trace and repeated exposures of Pb, through the environment or food, can develop into acute and chronic condition associated with neurological/intellectual impairment. Contamination sources of lead include: lead shot, oil, gasoline, crank cases, batteries, ceramics, and lead containing paints. Trace and repeated exposures of As, has been implicated in global diseases associated with skin, lung, and bladder cancers. Contamination sources of As include: soil, irrigation water, poultry feed additives, and pesticides. Small and backyard flocks, frequently free-range, can be exposed to these contaminants surrounding their environment. Ultimately, they can contribute to the total Pb and As burden in the food supply chain through poultry and egg products.

FDA regulatory limits for trace compounds are present for specific food products (e.g. Pb maximum levels of 0.05 ppm in juices and 0.10 ppm in candy). Similarly, EPA regulatory limits

in soil (Pb and As are 400 ppm and 0.40 ppm, respectively) and in drinking water (< 10 percent of sampling to exceed 15ppb for Pb and 0.010ppm for As) have been established. However, there is a lack of established Pb and As limits in shell eggs. The presence of Pb and As in shell eggs from non-commercially sourced shell eggs suggests the potential of public health risks, which necessitates further investigation.

4.3 Review of Literature

Although lead occurs naturally in the environment, it often occurs at higher levels in soils affected by human activity (Cornell, 2012). Some of these sources of lead contaminants (e.g. batteries, putty, asphalt products, leaded gasoline, spent oil, and lead shots) are found in urban living areas, while others are more commonly found in suburban or rural living environments.

According to Trampel and others (2003), lead contaminated eggs yolks and edible chicken meat from small and backyard flocks can pose a potential public health hazard, especially in children with repeated consumption. Lead consumption negatively affect young childrens' behavior, ability to learn, and optimal growth potential (Cornell, 2012). Trampel and others, 2003) focused on a small rural family farm in Iowa where twenty mixed-breed adult laying hens where clinically normal, but were exposed to chips of lead-based paint from their environment. Lead levels ranged from less than 0.05-0.76 ppm in the blood, 0.02-0.40 ppm in the egg yolks, and no detectable amount in the egg albumen. In the US, there are no health-based standards specifically for lead in chicken eggs; however, the US guidelines indicate lead levels in candy not exceed 0.10 ppm (FDA, 2006).

Splietthof and others (2014) evaluated lead concentrations in shell eggs within a New York urban setting. Lead was detected in 48% of eggs collected from the nine NYC henhouses

and was found as high as 167 μg/kg (equivalent to 0.167 ppm). According to recommendations by Cornell University and Cooperative Extension, lead in soil (typically ingested by chickens in chicken runs) can pose some risks even if test results are below guidance values (Cornell, 2012). If lead levels in eggs are found between 0.10 and 0.30 ppm there could be some increased risk for a child who frequently eats eggs with lead levels in this range (e.g. one egg a day over a long period of time). In these cases, Cornell Cooperative Extension (2012) recommends to add clean soil, mulch, or other clean cover material to the chicken run to reduce chickens' contact with and ingestion of lead in soil. They also recommend avoidance of scattering feed on bare ground in these areas (Cornell 2012).

In a study by Roegner and others (2013), the public health implications of two confirmed cases of lead poisoning in backyard chickens was of concern. Two cases, involving backyard chickens, illustrates one very important take-home message: lead exposures in backyard chickens may be chronic and subclinical (Roegner and others 2012). In the first case, a brown adult female chicken in a Santa Clarita, CA backyard flock, was found down and unresponsive. Toxicological evaluation found lead concentration was high in the liver, at 7.8 mg/kg wet weight (or 7.8 ppm) and consistent with exposure to or intoxication from lead. Eggs (mixed egg yolk and albumen) from this backyard flock presented lead levels at 72 μg/kg (or 0.072 ppm). In this case, lead toxicity was not suspected, but discovered only secondary to a toxicological assessment that included a heavy metal screen (Roegner and others, 2012). In another case, seven out of 15 backyard hens/chickens from El Dorado County, CA, died acutely without showing previous clinical signs. The flock, located in El Dorado County, CA, produced eggs well until a series of deaths lead to toxicological evaluations. Lead concentrations were found elevated in the liver at 5.4 mg/kg (or 5.4 ppm) wet weight (Roegner and others, 2012). These two

cases may not be isolated instances, but an indicator of the status of heavy metals that can be found in small and backyard flocks and their eggs. With the increased allowances to raise backyard chickens (within rural, suburban, and urban communities), comes a need for periodic screening of eggs or testing of blood levels of chickens (Roegner and others, 2012). This is particularly important in small flocks or back yard settings where birds have free access to a host of potential sources. In addition, client or public education about this potential hazard from backyard flocks should be addressed (Roegner and others, 2012).

4.3 Materials and Methods

4.3.1 Soil Samples

Environmental soil samples were collected from three sites within a small backyard flock habitat in rural Gadsden, AL. Pb and As analysis were conducted, via ICP-AES, according to EPA method 200.12 (Alabama Cooperative Extension System Soil, Forage, and Water Testing Laboratories, Auburn, AL).

4.3.2 Shell Egg Samples

Egg samples were randomly collected from a small backyard flock in rural Gadsden, AL. Twelve eggs were collected (in triplicates, at approximately 6 week intervals), totaling 36 eggs. Eggs were deshelled. Composites of 2 eggs units (yolk and albumen) were homogenized, which totaled 18 composite samples. Composite samples were analyzed for Pb and As, via ICP-MS, according to AOAC method 2013.16 (Eurofins, New Orleans, LA).

4.4 Results and Discussion

Environmental soil analysis, from three sites within a small backyard flock habitat, indicated trace compound concentrations of 45, 48, and 1,835 ppm for Pb and <0.1, <0.1, and 61 ppm for As. Pb and As values of 1,835 ppm and 61 ppm, were elevated comparative to EPA limits for clean-up in soils (Pb and As at 400 ppm and 0.40 ppm, respectively).

Mixture of the yolk and albumen analysis, from eggs of a small backyard flock, indicated Pb and As concentration ranges from 0.02 ppm to 0.05 ppm and <0.02 ppm to 0.04 ppm, respectively. In the absence of regulatory limits for Pb and As, specifically for shell eggs, it is important to comparatively consider existing EPA permissible level for As at 0.010 ppm in drinking water, as well as the FDA maximum Pb level of 0.05 ppm in juices and 0.10 ppm in candy. Analyzed samples were below or proximate to regulatory limits for Pb. Regarding As values, the majority were below regulatory limits; however, several exceeded EPA standards.

4.6 Conclusions

The presence of Pb and As in eggs from a small backyard flock can be an indicator of potential chemical safety risks in consuming eggs from non-commercial sources. In this study, the presence of Pb and As suggest there is a potential safety risk, especially when combined with the growth in distribution channels for non-commercial sources of shell egg. Further investigation, specifically with an expansion of geographic sampling, is necessary to comprehend the scope of public health concern.

CHAPTER V: EGG QUALITY OF NON-COMMERCIAL SHELL EGGS

5.1 Abstract

A growing interest in non-commercially sourced shell eggs is fueled by consumer perception of higher quality. Due to the marginal data available these shell eggs, this study evaluated the Haugh unit, shell strength, vitelline membrane strength, and yolk color, to establish baseline information. Yolk color data (n=1,118) indicated an average digital yolk color (Digital YolkFan™) of 9.0. Haugh unit (HU) data (n=1273) indicated an average of 75.24 HU. Based on collected HU values and equivalence to USDA grading, approximately 65% of all samples evaluated were Grade AA, 25% were Grade A, and 10% were Grade B or less. Average shell strength (n=1,273) was observed at 3,939g Force, which is within the average range of commercially produced brown and white eggs. Average vitelline membrane strength (n=1,191) was observed at 122.79g Force, which is below previously evaluated commercial eggs that underwent extended storage. Based on this study, the quality of shell eggs from non-commercial sources can vary from comparable to retail markets to well below.

5.2 Introduction

Consumer preferences for shell eggs non-commercial sources originates from perceptions of higher quality and freshness. Ideally, decreased distribution time between shell egg producers and consumer point-of-sale lends itself to a product that is higher in quality. Unfortunately, unknown variables in handling, storage, and transportation associated with small and backyard flocks potentially affect the quality of shell eggs. Quality parameters of shell eggs have been evaluated by: albumen height, Haugh unit, static compression shell strength, static compression vitelline membrane strength, yolk color, and shell dynamic stiffness. Egg quality deterioration is

a factor of time, temperature, humidity, and handling (Stadelman and Cotterill, 1995). Literature on quality assessment of shell eggs in the United States has been focused on variable: poultry housing units (Karcher and others 2015), litter composition (Oke and others 2014), poultry nutrition (Karcher and others 2015), and pasteurizing techniques (Geveke and others 2016; Caudill and others, 2010). Although some literature on quality of shell eggs from small and backyard flocks have been observed in developing countries (Hussain and others 2013; Desalew 2014), data on these non-commercial suppliers in the United States does not appear available. Quality assessment of shell eggs from non-commercial shell eggs can validate/nulify consumer perceptions as well as identify potential points of interest in egg handling practices.

5.2 Review of Literature

5.2.1 Yolk Color

Color often influences our perception of food, and can be a significant aspect of food quality. Sensory evaluation is commonly used as an instrument for assessment of food color; and the DSM Yolk Color Fan (formerly Roche Yolk Color Fan) has been the main index for egg yolk color evaluation. Although consumer preferences surrounding egg yolk color vary by geographic region, many parts of the world value deeply colored, orange toned yolks; however, deeper yolk colors in shell eggs does not necessarily have a direct correlation to a higher quality shell egg (Beardsworth and Hernandez, 2004). Yolk pigmentation is directly related to carotenoids in the nutrient intake of layer flocks, and has easily been modified by feed ingredients (Beardsworth and Hernandez, 2004). Relative to commercially produced eggs, non-commercial shell eggs have a potential for a high degree of variation in nutrient intake (e.g. brand/formulation of feed; environmental vegetation, soil, and insects; access to kitchen scraps) which can produce a

wide range of yolk color.

5.2.2 Vitelline Membrane Strength

The vitelline membrane is the translucent sheath that encloses the yolk of the hen's egg, separating the yolk from the egg white. The yolk membrane of a hen's egg is largely composed of two distinct layers of different compositions and structures, the inner layer (lamina perivitellina), and the outer layer (lamina extravitellina) (Bellairs and others 1963). Vitelline membrane characteristics are associated with physical quality factors as well as microbial quality factors in shell eggs (Gast and Holt, 2001; Jones, 2006). Vitelline membrane properties are dependent on length and conditions of shell egg storage (Smolinska and Trziszka 1982; Jones, 2006). Jones and others (2002 and 2005) have indicated vitelline membrane strength has been found to decrease during prolonged cold storage. In addition, Jones and others (2006) concluded the vitelline membrane elasticity also decreased during prolonged storage, which could lead to yolks more easily rupturing as consumers crack the eggs. More recently, Jones and others (2018) evaluated commercially produced shell eggs which were: washed; washed, oiled; and unwashed shell eggs. After a 6 week period, observed average vitelline membrane strengths for washed (4 ºC); washed, oiled (4 ºC); unwashed (4 ºC); and unwashed (22 ºC); were 155.7, 151.7, 155.0, and 129.9 g Force, respectively.

5.2.3 Haugh Unit (HU)

Haugh (1937) developed the HU measurement for interior shell egg quality. HU measurement accounts for the egg weight and corrects for all eggs to be compared,

through mathematical manipulations, regardless of the actual weight of the egg. Specifically, it is a correlation of albumen height and egg weight and calculated as $HU=100 \log (h+7.57-1.7w^{0.37}+7.6)$, where (h) is the albumen height and (w) is the weight of the shell egg. Shell egg USDA Graded AA and A, commonly found in retail markets, correlate to >72 HU and 72-60 HU, respectively. Although various methods have been utilized to assess shell egg interior quality, the HU has revered as the "gold standard" according to Jones (2012).

Shell egg studies have shown correlations, observations, and specific factors affecting HU of shell eggs. Curtis and others (1985) have observed greater HU in brown egg layers (85.11HU) relative to white egg layers (81.92HU). Under non-comparable conditions, Jones and others (2010) observed a higher HU in the traditional white eggs (84.42) compared to traditional brown eggs (79.08). Silversides and others (1993) observed negative changes in egg weight as a hen ages. Additionally, Silversides (1994) reported a linear decrease in albumen height as a hen ages. Consequently, hen age has been found to be a factor in HU values. Several studies established that HU decreases as the hen ages (Cunningham and others 1960; Curtis and others 1985; Izat and others 1986). Doyon and others (1986) reported HU and albumen height decrease at a constant rate as the hen ages. In addition, Doyon and others (1986) observed HU decreased at a rate of 0.0458 units/day of lay (stored at 15.5-20 ℃; 13.15 HU over 287 days). Furthermore, Jones and others (2018) evaluated various wash, wash/oiled, and unwashed shell eggs at refrigerated (4 ℃) and room temperature (22 ℃) storage conditions. After one week of storage, observations concluded all refrigerated treatments had HU scores of > 83, correlating to USDA Grade AA; whereas, room temperature eggs had scores at or

below the minimum for USDA Grade A classification.

5.2.4 Shell Strength

Egg shells are the natural packing material for the egg contents. Shell strength is important for preservation of the egg's quality, as well as its safety for consumption, specifically from attack of microbial pathogens (Mertens and others, 2006). Shell strength studies have focused on commercial flocks. Anderson and others (2004) found that as the hen ages, shell breaking strength decreased as did percent shell and specific gravity. Jones and others (2010) observed traditional housed brown eggs had greater shell strength (4,314 g Force) than traditional housed white eggs shell strength (3,409.16 g Force). In addition, Jones and others (2010) observed brown shelled eggs from free-roaming hen environments were also higher in shell strength (4,165.85 g Force) relative to the traditional white eggs. In a study evaluating the impact of egg washing and storage, Jones and others (2018) observed that shell strength was not influenced by washing treatments or week of storage.

5.3 Materials and Methods

5.3.1 Sampling

Convenience random sampling of shell eggs (n=1,273) from 52 small flocks, throughout the state of Alabama (Figure 5.1). Sampling sources included: backyard flocks; health food stores, small farms, and farmers markets. Upon obtaining samples, shell eggs were transported in coolers with frozen gel packs and stored at 7 ± 2 ℃ for 48 h prior to evaluations.

5.3.2 Egg Weight, Albumen Height, and Haugh Unit (HU) Measurements

Upon removal from storage, shell eggs were inspected and cracked samples were discarded/eliminated. Shell eggs were evaluated for egg weight, albumen height, and HU (n=1,273). Upon daily receipt from suppliers, shell eggs were stored at 7 ± 2 ℃ for 24 h prior to evaluation. Shell eggs were weighed and gently deshelled onto a leveled smooth platform. Each egg albumen height (millimeter) was measured with a tripod micrometer (S-6428, B.C. Ames, Inc., Melrose, MA) at 3 locations, and with extreme care to detect the critical point at which the tip just touches the albumen. Triplicate values of albumen heights were averaged. HU were calculated with the formula $HU=100 * \log (h+7.57-1.7w^{0.37}+7.6)$, where (h) was the averaged albumen height and (w) was the weight of each egg (Haugh 1937).

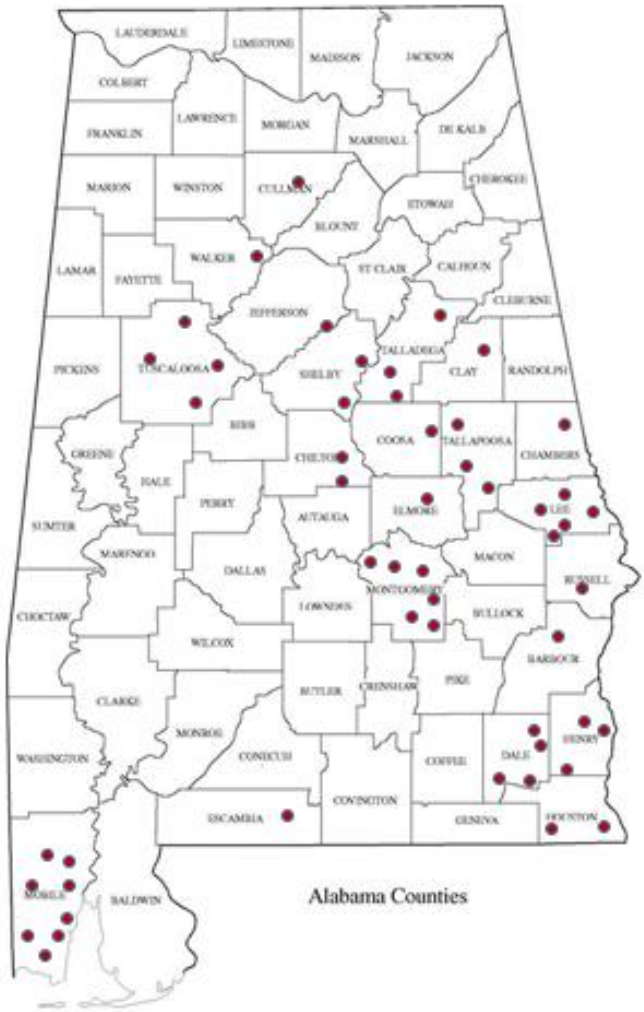

Figure 5.1: Non-commercial shell egg sampling sites for egg quality evaluation (n=52)*
***** Yolk color evaluation excludes Escambia county site

5.3.3 Shell Strength Measurements

Shell static compression strength (n=1,273) was measured according to Jones and others (2010 and 2017). A texture analyzer (TA-XT2plus; Texture Technologies, Scarsdale, NY) equipped with a 5-kg load cell (calibrated with a 2 kg weight) and a 3-in. diameter aluminum compressions disc (TA-30, Texture Technologies) was utilized. The shell egg was presented on its side in an egg holder with posts (TA-650, Texture Technologies, Scarsdale, NY). Test parameters included a speed of 2 mm/s and a trigger force of 0.001 kg. Direct pressure was applied to the shell egg until an initial fracture. The shell strength was recorded as grams of force required for an initial fracture of the egg shell. The force required to break the vitelline membrane corresponds to its strength, and a stronger shell requires more force to break (Jones and others, 2010 and 2017).

5.3.4 Vitelline Membrane Strength Measurements

Upon completion of Haugh unit evaluation, the yolk was separated and placed in a disposable 100 X 15 mm Petri dish for vitelline membrane strength evaluation (n=1,191). Vitelline membrane strength was evaluated by static compression with a TA-XT2i texture analyzer (TA-XT2plus; Texture Technologies, Scarsdale, NY) equipped with a 500 g load cell (calibrated using a 200 g weight), and a 3-in. diameter aluminum compressions disc (TA-30, Texture Technologies, Scarsdale, NY). Test parameters were as follows: 1 mm/s test speed, 5-g trigger force, and 2-mm trigger distance. Direct pressure was applied to the yolk until the vitelline membrane ruptured. The vitelline membrane breaking strength was recorded as grams of force required to rupture the

membrane. The force required to break the vitelline membrane corresponds to its strength, and a stronger membrane requires more force to break (Jones and others, 2010 and 2018).

5.3.5 Yolk Color

Similar to HU, shell strength, and vitelline membrane strength, convenience random sampling of shell eggs (n=1,118) from 51 small flocks, throughout the state of Alabama, were evaluated for yolk color (Figure 5.1). Historically, a manual color fan (color range between 1 and 15) has been utilized for assessment of yolk color; however, this evaluation utilized a novel yolk colorimeter (Digital YolkFan™, Nix, Ontario Canada) to minimize subjectivity and other potential sources of variation. Upon removal from storage, shell eggs were inspected, and cracked samples were discarded/eliminated. Upon deshelling, egg contents were emptied onto a disposable 100 X 15 mm Petri dish. The Digital YolkFan™ was placed directly over the yolk and readings were documented.

5.4 Results and Discussion

5.4.1 Yolk Color

Overall yolk color data (Figure 5.2) indicates an average digital yolk color of 9.0 with a 95% CI = [8.9,9.1] yolk color. Yolk color frequency distribution (Figure 5.3) indicates the majority of samples evaluated included: 350 samples (9 yolk fan color) and 246 samples (10 yolk fan color) at 31.3% and 21.0%, respectively. Upper yolk color values of 11, 12, 13, and 14 corresponded to 8.1%, 6.0%, 1.4%, and 0.4%, respectively, of total

samples. Lower yolk values of 2, 3, 4, 5, 6, 7, and 8 corresponded to 0.2%, 0.5%, 0.3%, 1.7%, 4.7%, 13.4%, and 10%, respectively, of total samples. Collectively, approximately 69% of all samples evaluated had a yolk color of ≥9. Normality probability plot (Figure 5.4) shows R^2=0.9896., indicating a highly normal distribution.

Yolk color data by supplier (n=51) (Figures 5.5) indicates an average digital yolk color of 8.97 with a 95% CI = [8.5,9.5] yolk color. Yolk color frequency distribution (Figure 5.6) indicated the majority of suppliers' average yolk colors were: 10 suppliers (8 yolk fan color); 26 suppliers (9 yolk fan color) and 9 suppliers (10 yolk fan color) at 19%, 51.0%, and 18%, of total samples, respectively. Additional supplier average yolk digital color values of 7 and 11 each corresponded to 6% of total samples. Normality probability plot (Figure 5.7) shows R^2=0.9826, indicating a highly normal distribution.

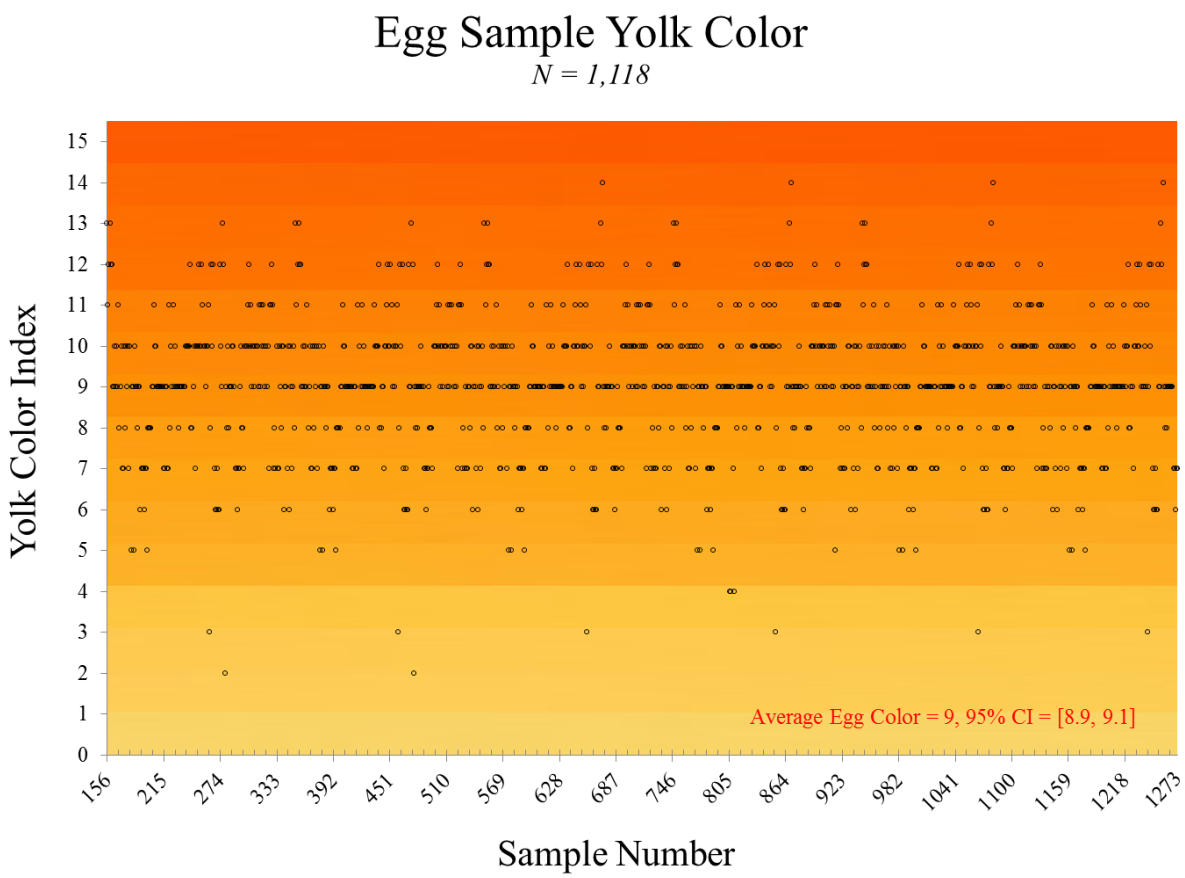

Figure 5.2: Yolk color scatter plot of non-commercial shell eggs. n=1,118. Average yolk color of 9.0 with a 95% Confidence Interval (CI) = [8.9, 9.1] yolk color index.

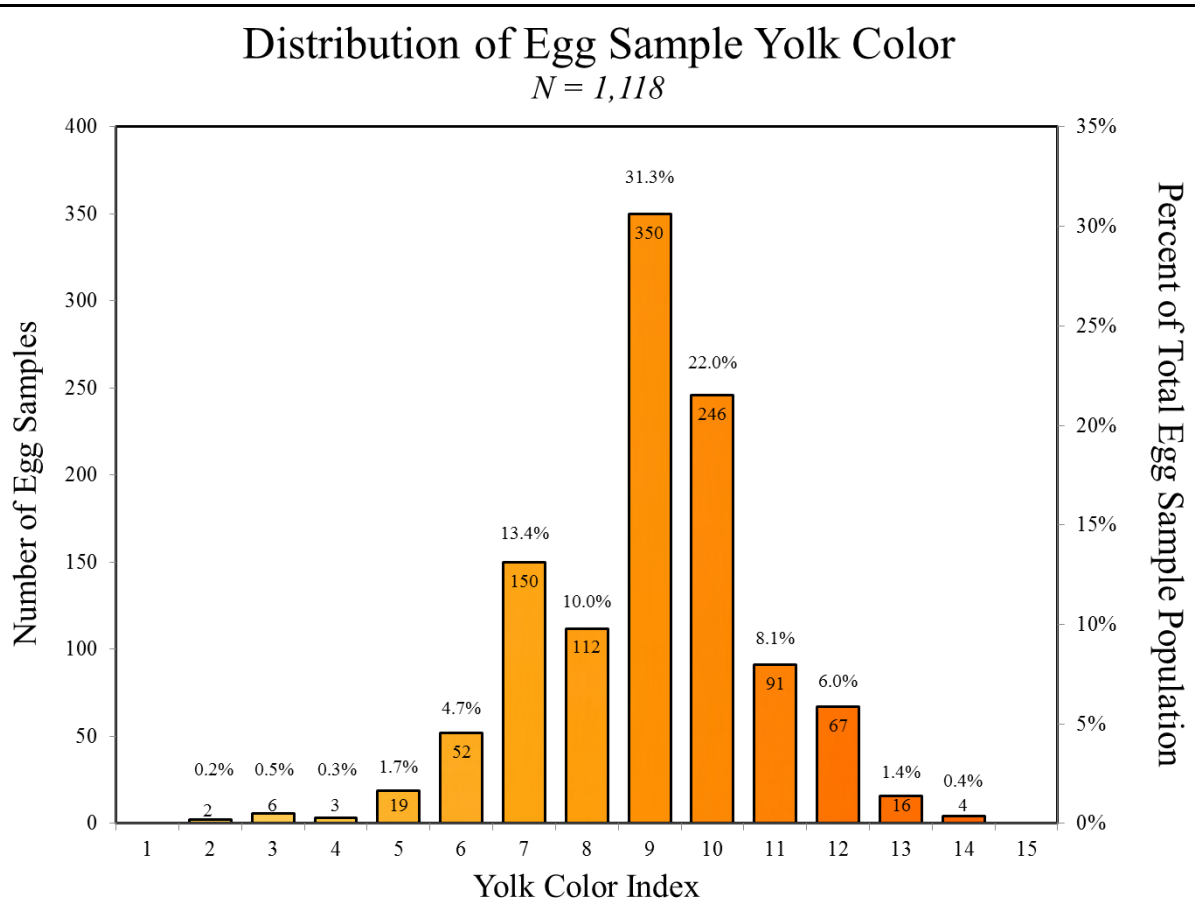

Figure 5.3 Yolk color frequency distribution histogram of non-commercial shell eggs.

n=1,118.

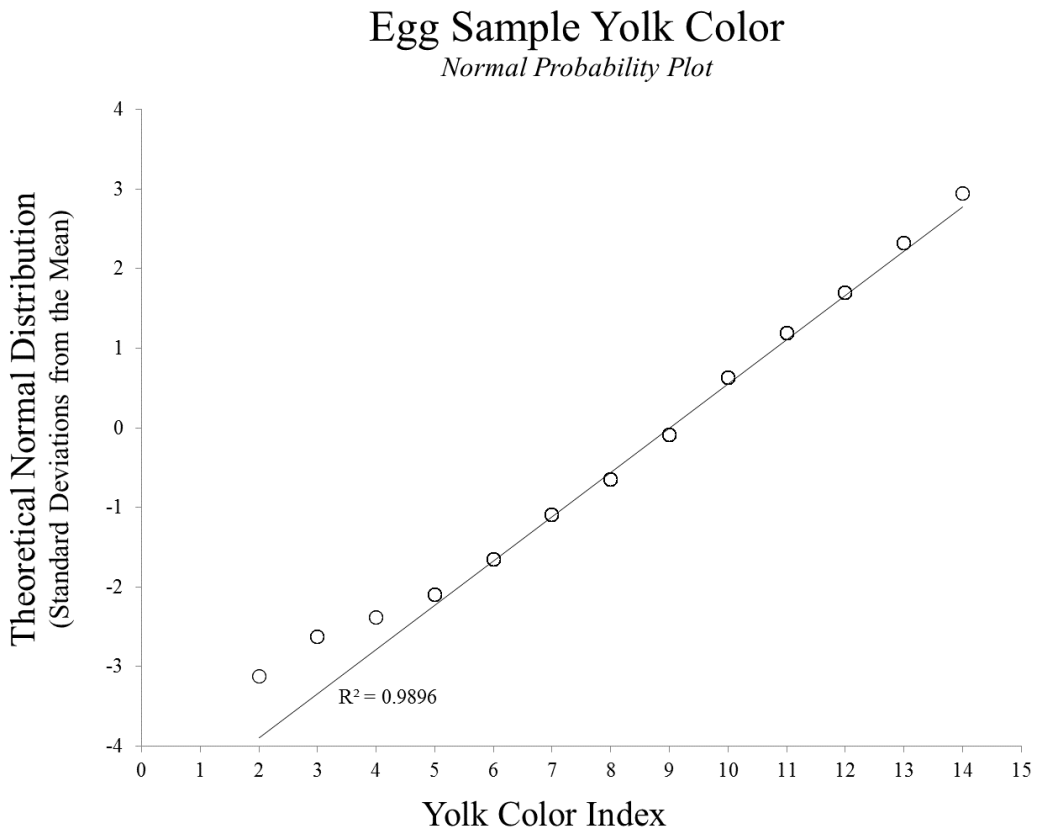

Figure 5.4 Yolk color normality probability plot of non-commercial shell eggs. n=1,118. R^2=0.9896.

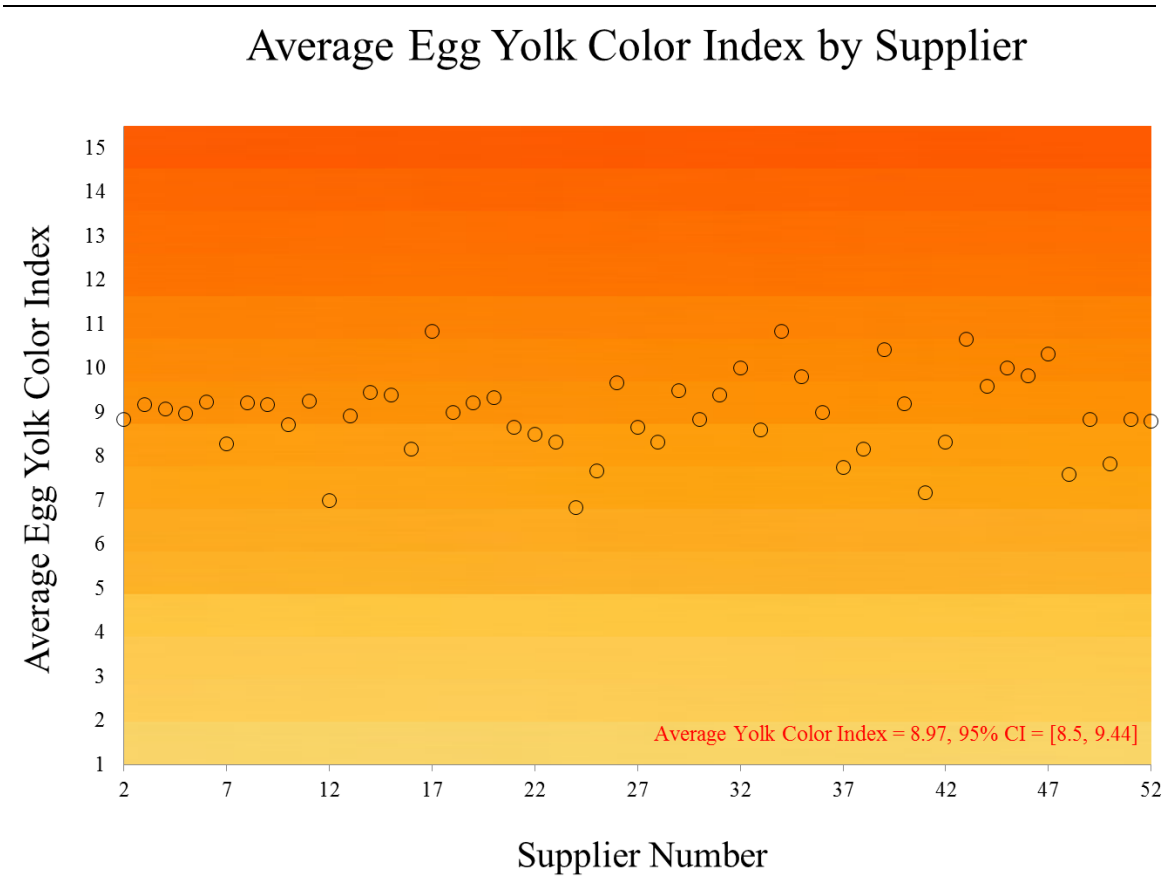

Figure 5.5: Yolk color scatter plot of non-commercial shell eggs by supplier. n=51. Average yolk color of 8.97 with a 95% Confidence Interval (CI) = [8.5, 9.44] yolk color index.

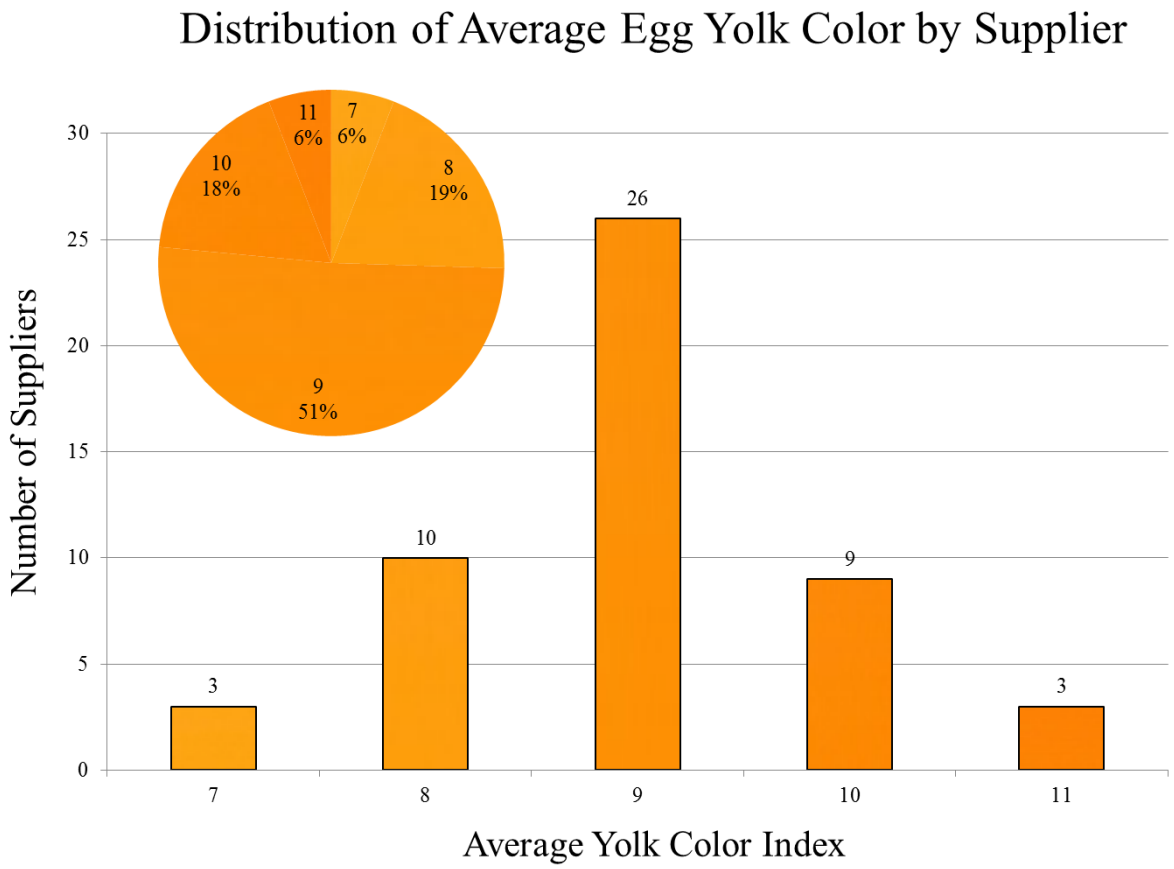

Figure 5.6: Yolk color frequency distribution histogram of non-commercial shell egg by supplier. N=51.

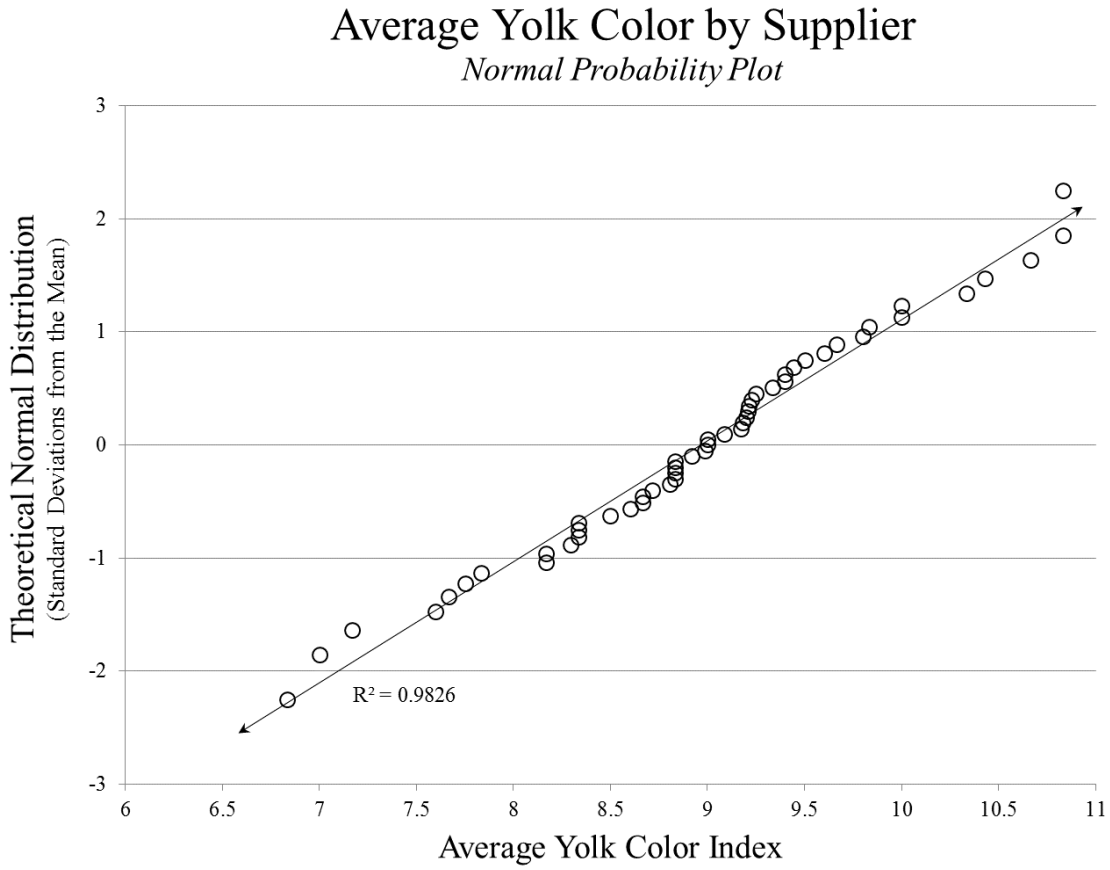

Figure 5.7: Yolk color normality probability plot of non-commercial shell eggs by supplier.

N=51. R^2=0.9826

5.4.2 Egg Weight, Albumen Height, and Haugh Unit

Egg weight data (n=1,273) (Figures 5.8) indicates an average weight of 57.06 g (comparable to commercially produced eggs) and a 95% Confidence Interval (CI) = [56.66, 57.45] g. Egg weight frequency distribution (Figure 5.9) indicates the majority of samples weighed between 53 up to 58 g (369 samples) and 58 up to 63 g (370). Weight normality probability plot (Figure 5.10) indicates a normal distribution of egg weights with R^2=0.9753.

Albumen height data (n=1,273)(Figure 5.11) indicates an average albumen height of 5.85 mm with a 95% CI = [5.76, 5.94]mm. Albumen height frequency distribution (Figure 5.12) indicates the majority of samples obtained measured between 5 up to 6 mm (347 samples) and 6 up to 7 mm (306 samples). Normality probability plot (Figure 5.13) indicates a normal distribution of albumen height with R^2=0.9951.

Overall, Haugh unit (HU) data (n=1,273) (Figures 5.14) indicates an average HU of 75.24 with a 95% CI = [74.56, 75.93] HU. HU frequency distribution (Figure 5.15) indicates the majority of samples obtained included: 316 samples (60 up to 72 HU); 364 samples (72 up to 80 HU); 351 samples (80 up to 90HU); and 117 samples (90-100HU). HU measurements above 72 HU corresponds to Grade AA shell egg standards, according to USDA (2000). Figure 5.15 also illustrates, approximately 65% of all samples evaluated were Grade AA, 25% were Grade A, and 10% were Grade B or less. Considering Grade B eggs are not typically found in retail stores, potentially due to their lack of their marketability, the overall 10% frequency of Grade B shell eggs from non-commercial sources may be a cause for concern with consumers.

Average HU by supplier (n=52) (Figure 5.16) indicates an average value of 70.23 HU with a 95% CI = [67.43, 73.03] HU from non-commercial shell eggs by supplier. HU frequency distribution by supplier (Figure 5.17), indicates approximately 50% of suppliers provided shell eggs with an average Grade AA, 33% were Grade A, and 17% Grade B or less. Considering Grade B eggs are not typically found in retail stores, potentially due to their lack of their marketability, the 17% frequency of Grade B shell eggs from non-commercial sources by supplier may be a cause for concern with consumers.

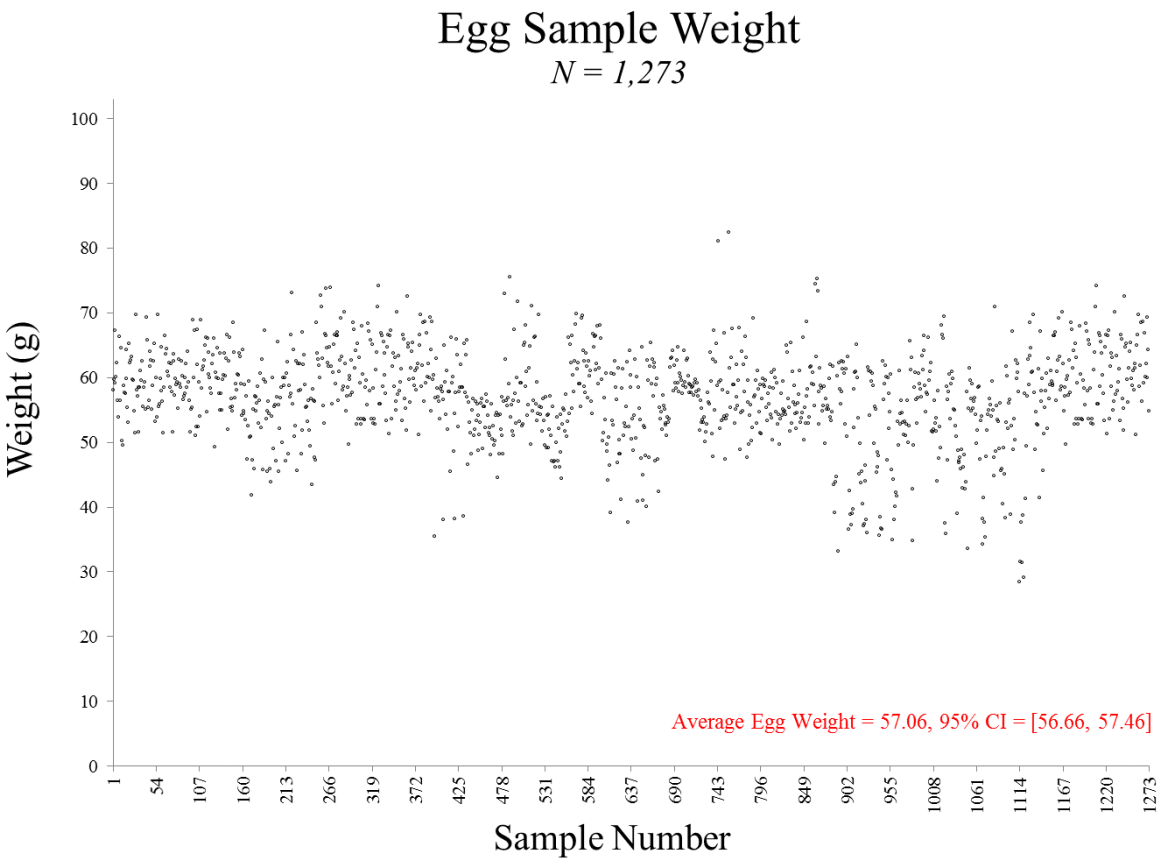

Figure 5.8: Weight scatter plot of non-commercial shell egg. n=1,273. Average weight of 57.06 g with a 95% Confidence Interval (CI) = [56.66, 57.46] g.

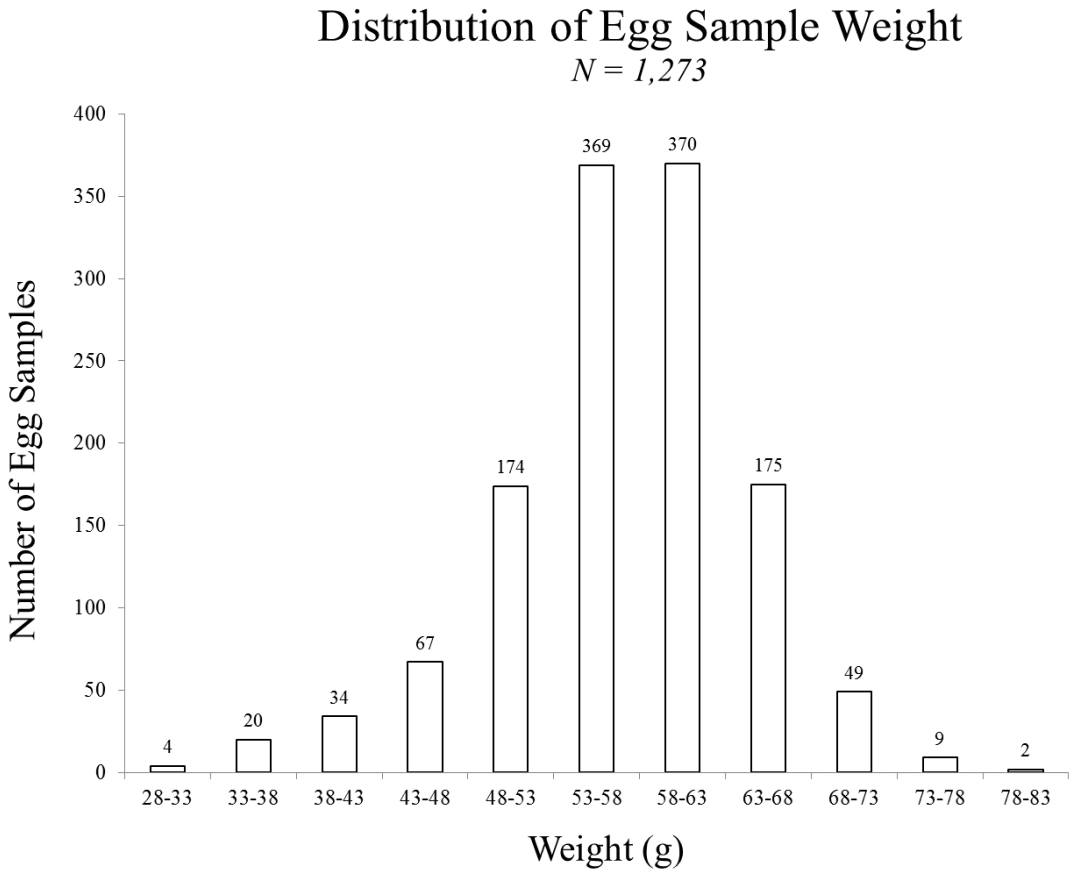

Figure 5.9: Weight frequency distribution histogram of non-commercial shell egg. n=1,273.

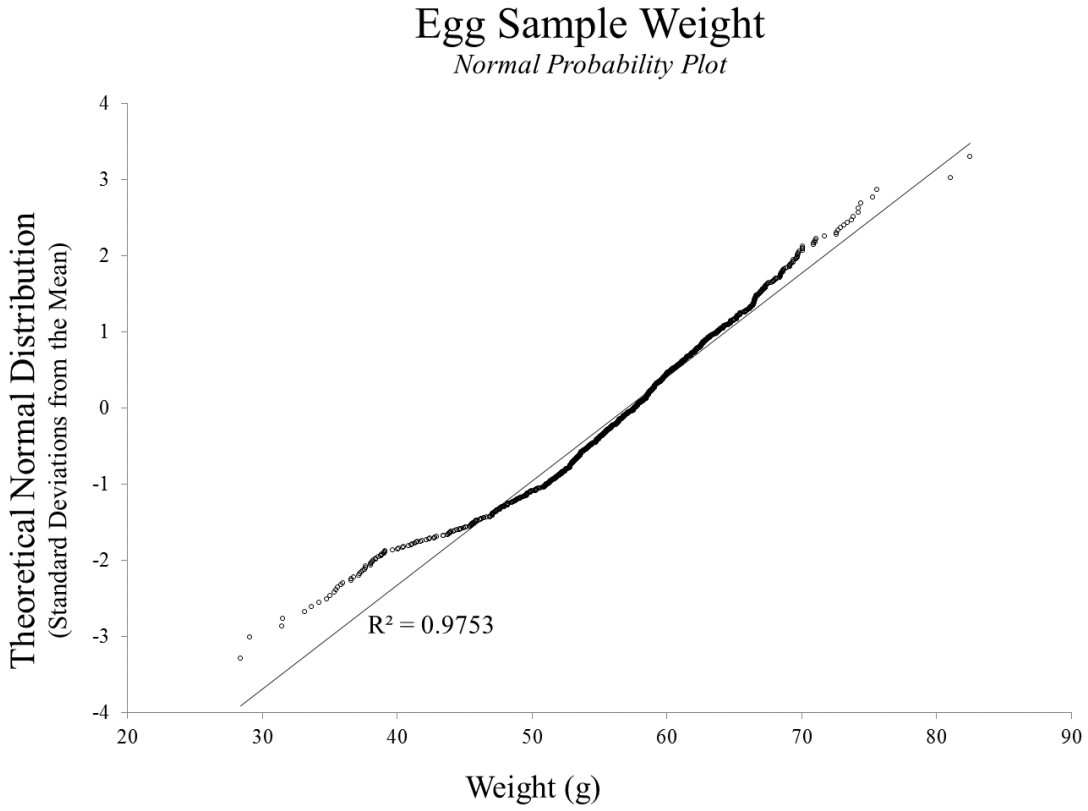

Figure 5.10: Weight normality probability plot of non-commercial shell egg. N=1,273. R^2=0.9753.

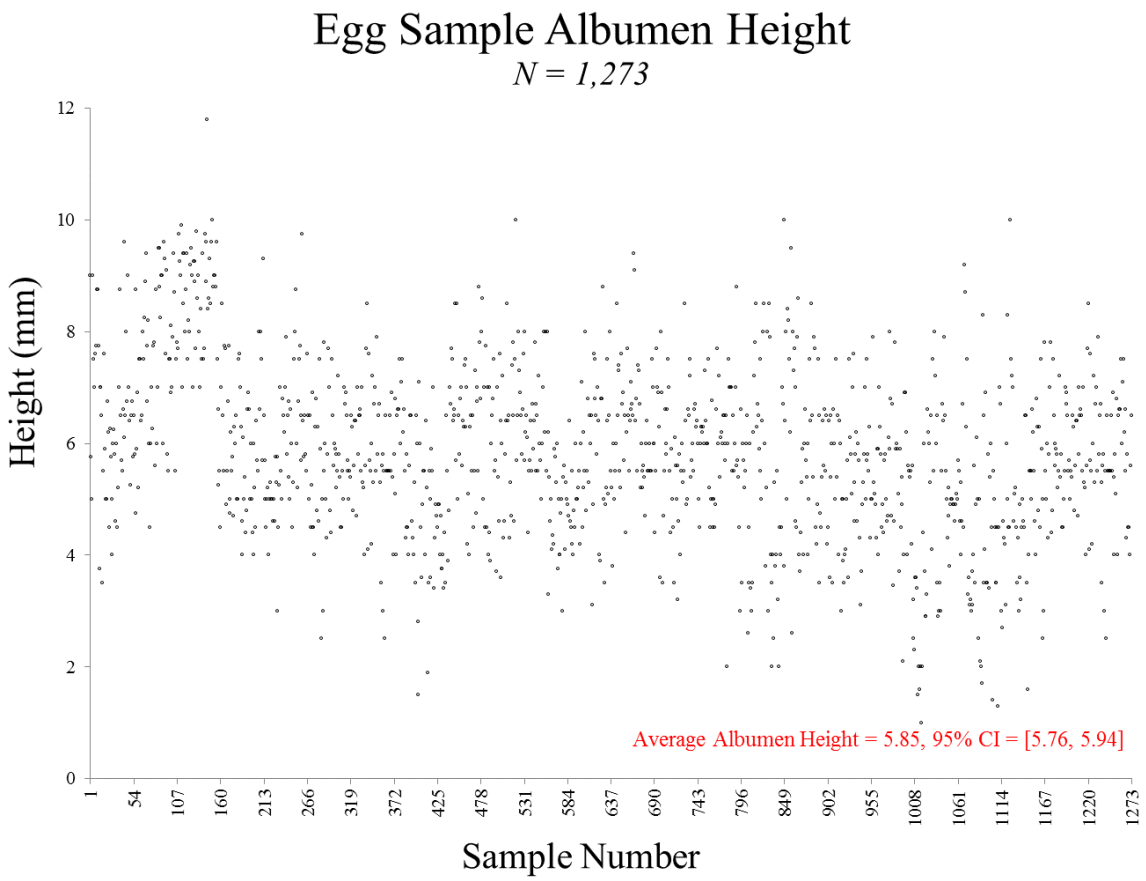

Figure 5.11: Albumen height scatter plot of non-commercial shell egg. n=1,273. Average albumen height of 5.85 mm with a 95% Confidence Interval (CI) = [5.76, 5.94] mm.

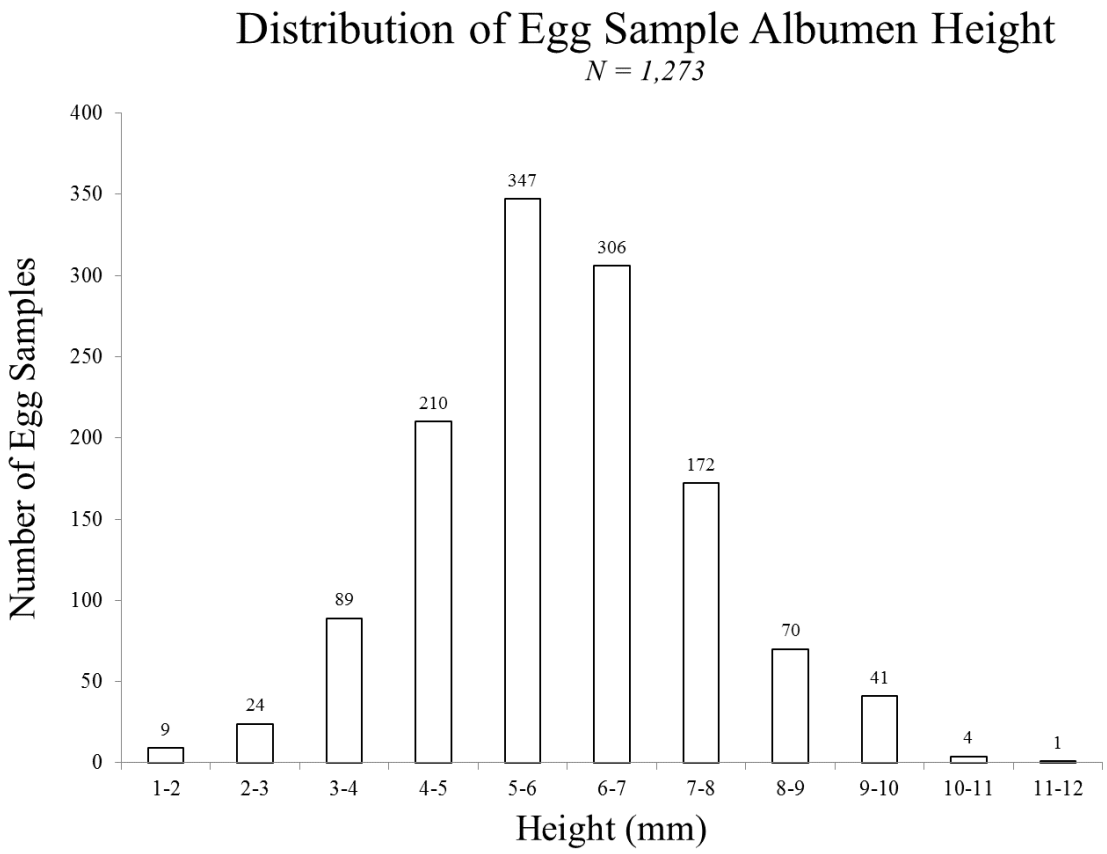

Figure 5.12: Albumen height frequency distribution histogram of non-commercial shell eggs. n=1,273.

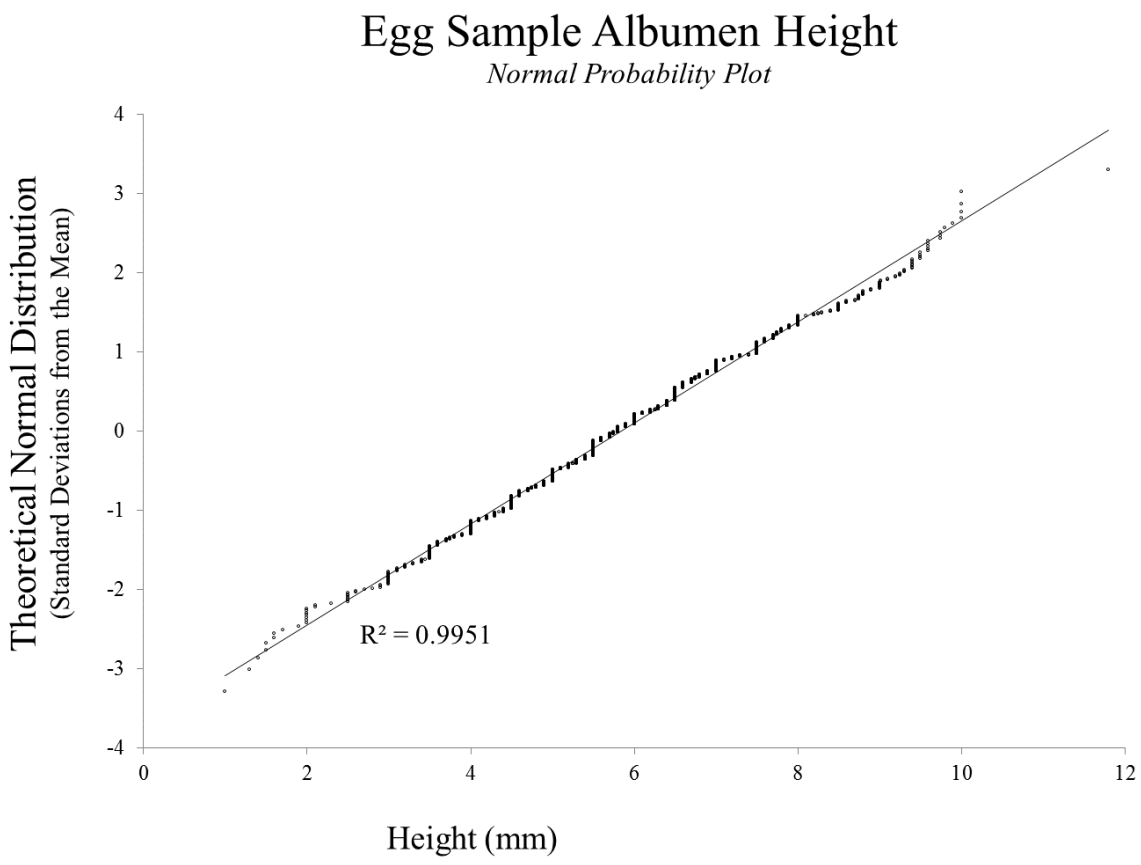

Figure 5.13: Albumen height normality probability plot of non-commercial shell eggs.

n=1,273. R^2=0.9951.

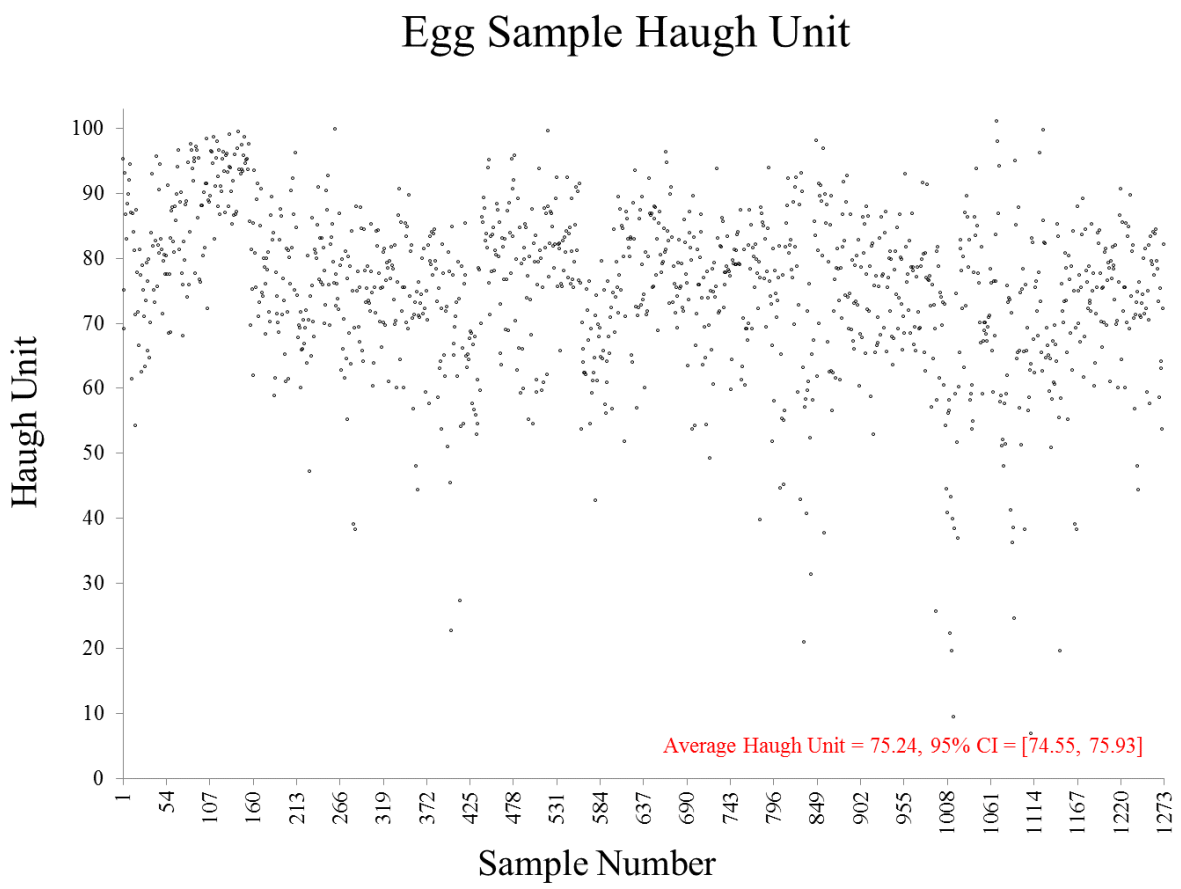

Figure 5.14: Haugh Unit (HU) scatter plot of non-commercial shell eggs. n=1,273; Calculated as H.U =100 log (h+7.57−1.7w0.37+7.6), where (h) was the averaged albumen height and (w) was the weight of each egg. Average of 75.24 HU with a 95% Confidence Interval (CI) = [74.55, 75.93] HU.

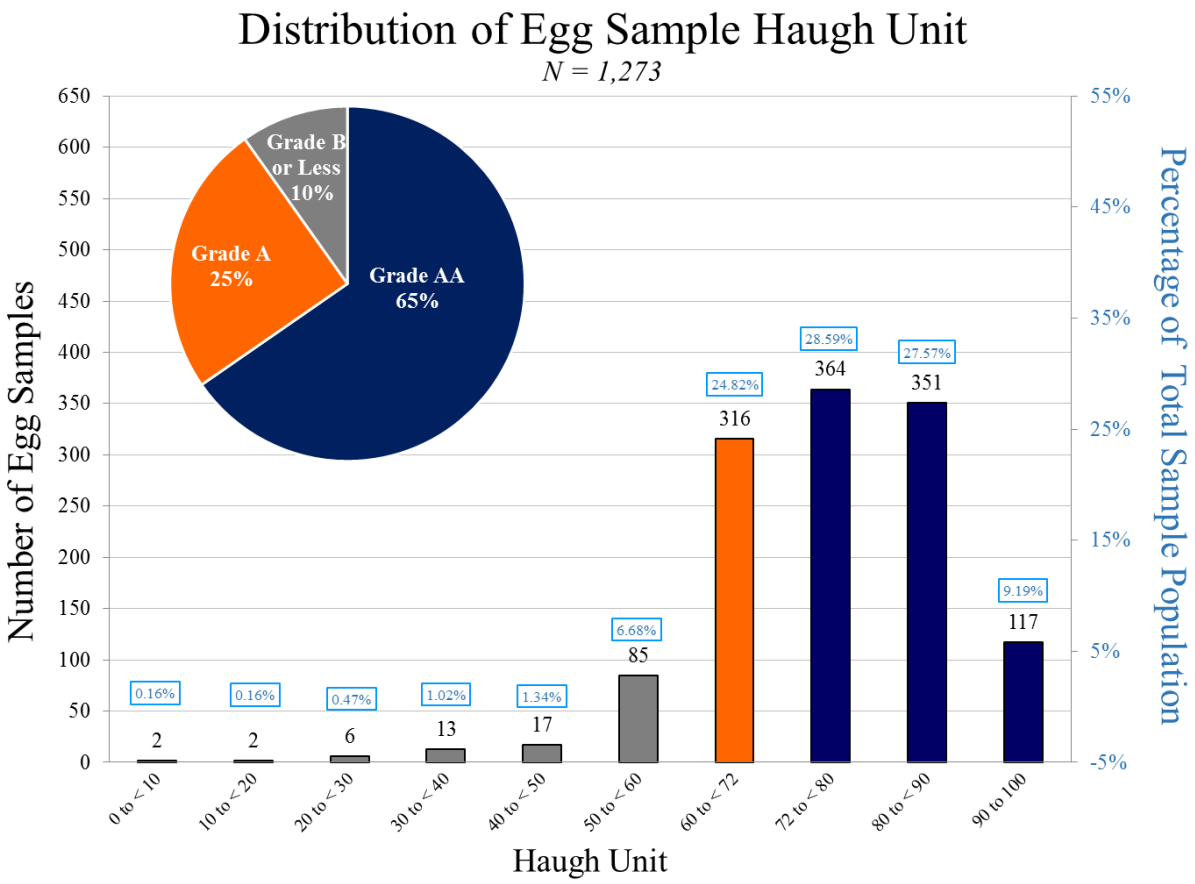

Figure 5.15: Haugh Unit (HU) frequency distribution histogram of non-commercial shell eggs. n=1,273

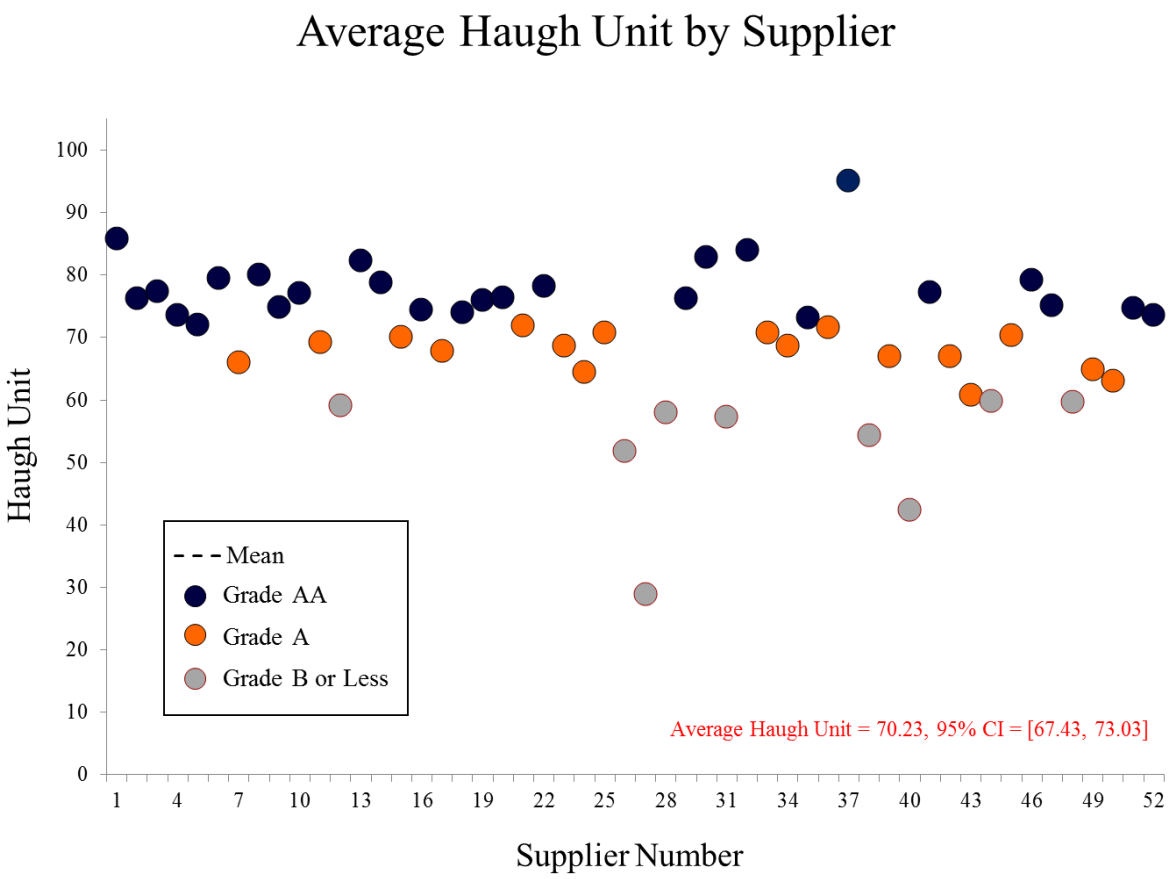

Figure 5.16: Haugh Unit (HU) scatter plot of non-commercial shell eggs by supplier. n=52.

Average of 70.23 HU with a 95% Confidence Interval = [67.42, 73.03] HU.

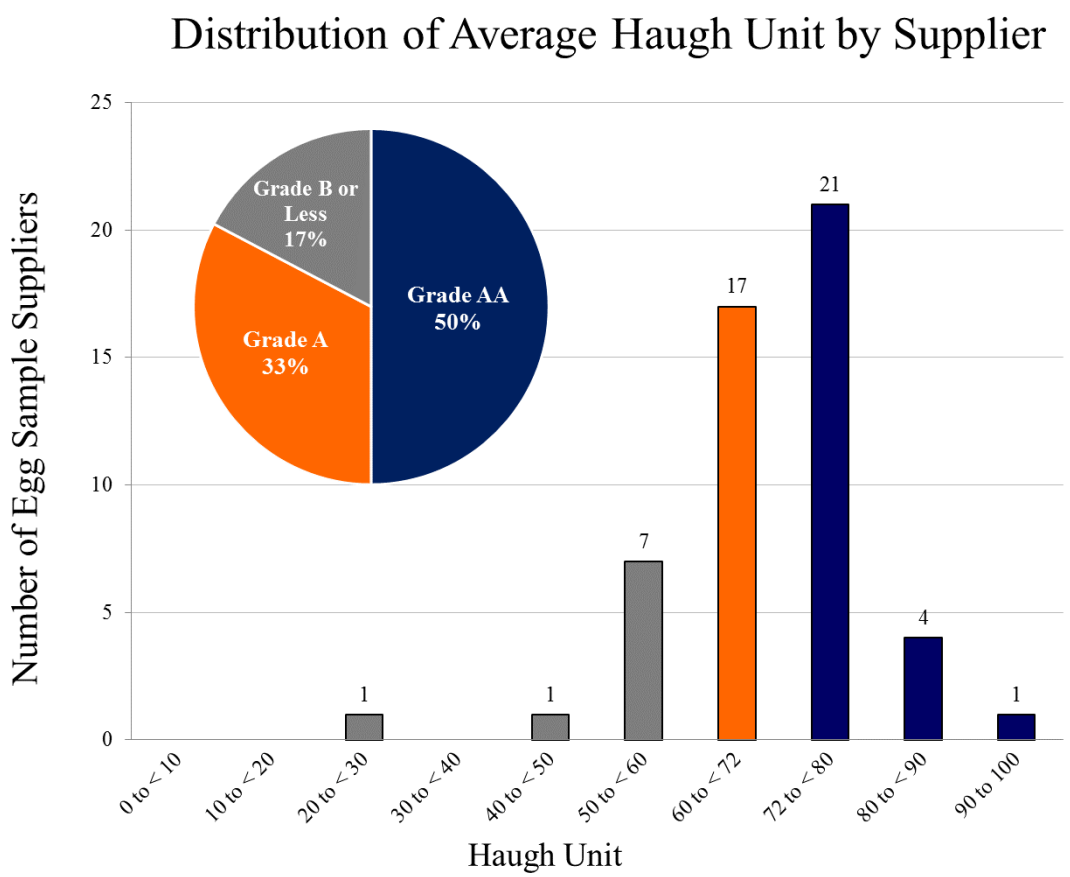

Figure 5.17: Haugh Unit (HU) frequency distribution histogram of non-commercial shell eggs values by supplier. N=1,273

5.4.3 Shell Strength

Overall shell strength data (Figures 5.18) indicates an average shell strength of 3,940g Force with a 95% CI = [3,892, 3,987] g Force. Comparative to average shell strength of commercially evaluated shell eggs by Jones and others (2010), these samples were weaker than brown eggs (4,130.61 g Force) and stronger than white eggs (3,690.31g Force). This mid-range of shell strength can be attributed to the combination of brown and white eggs received for sampling in this study. Shell strength frequency distribution (Figure 5.19) indicates the majority of samples evaluated included shell strength of: 213 samples (3000 up to 3500g Force); 306 samples (3,500 up to 4,000 g Force); and 259 samples (4000 up to 4500 g Force). Normality probability plot (Figure 5.20) shows R^2=0.9969, indicating a highly normal distribution.

Shell strength data, by supplier (Figures 5.21), indicates an average shell strength of 3,951 g Force with a 95% Confidence Interval (CI) = [3,727, 4,175] g Force. Shell strength frequency distribution (Figure 5.22) indicated the majority of suppliers' average shell strength were: 25 suppliers (3,500 up to 4,000 g Force) and 15 suppliers (4,000 up to 4,500 g Force). Figure 5.23 illustrates the shell strength normality probability plot of non-commercial shell eggs by supplier (R^2=0.9798), indicating a highly normal distribution.

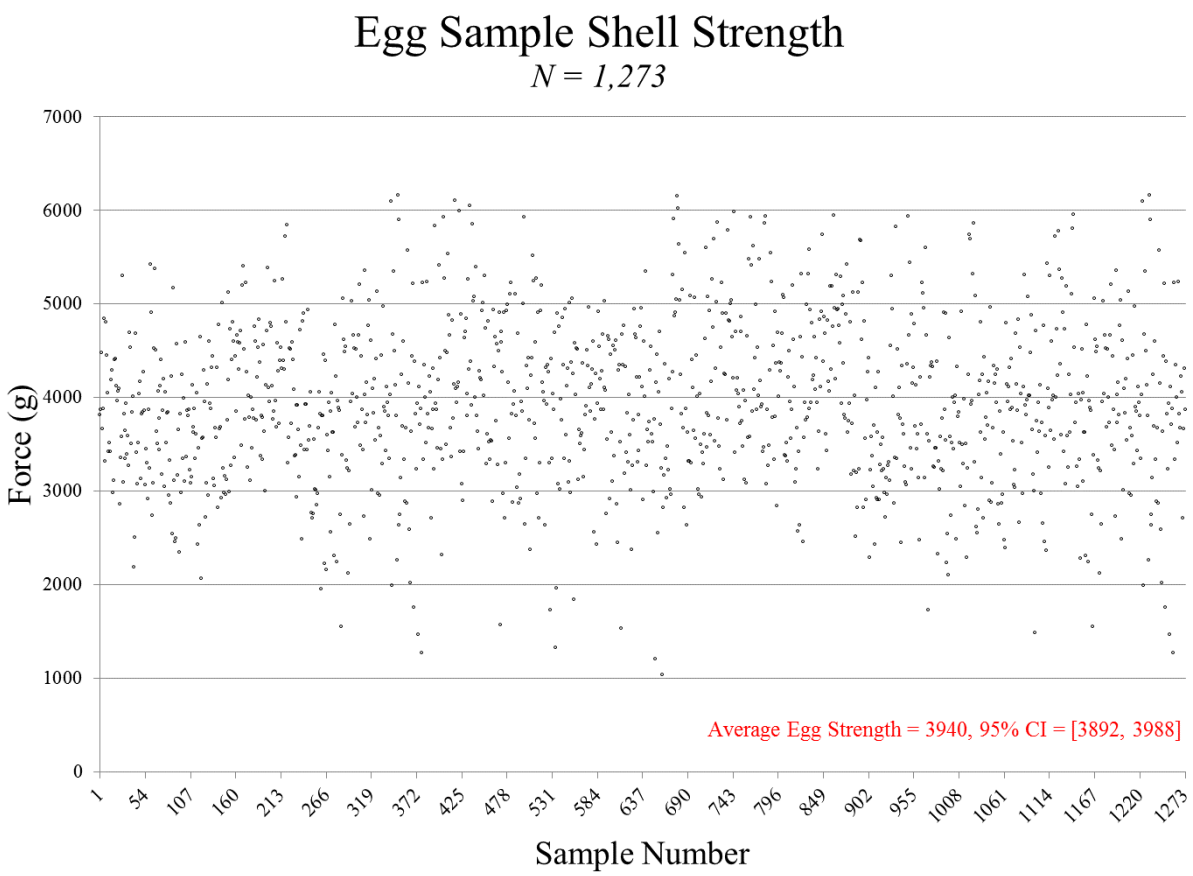

Figure 5.18: Shell strength scatter plot of non-commercial shell eggs. N=1,273. Average shell strength of 3,940 g Force with a 95% Confidence Interval (CI) = [3,892, 3,988] g Force.

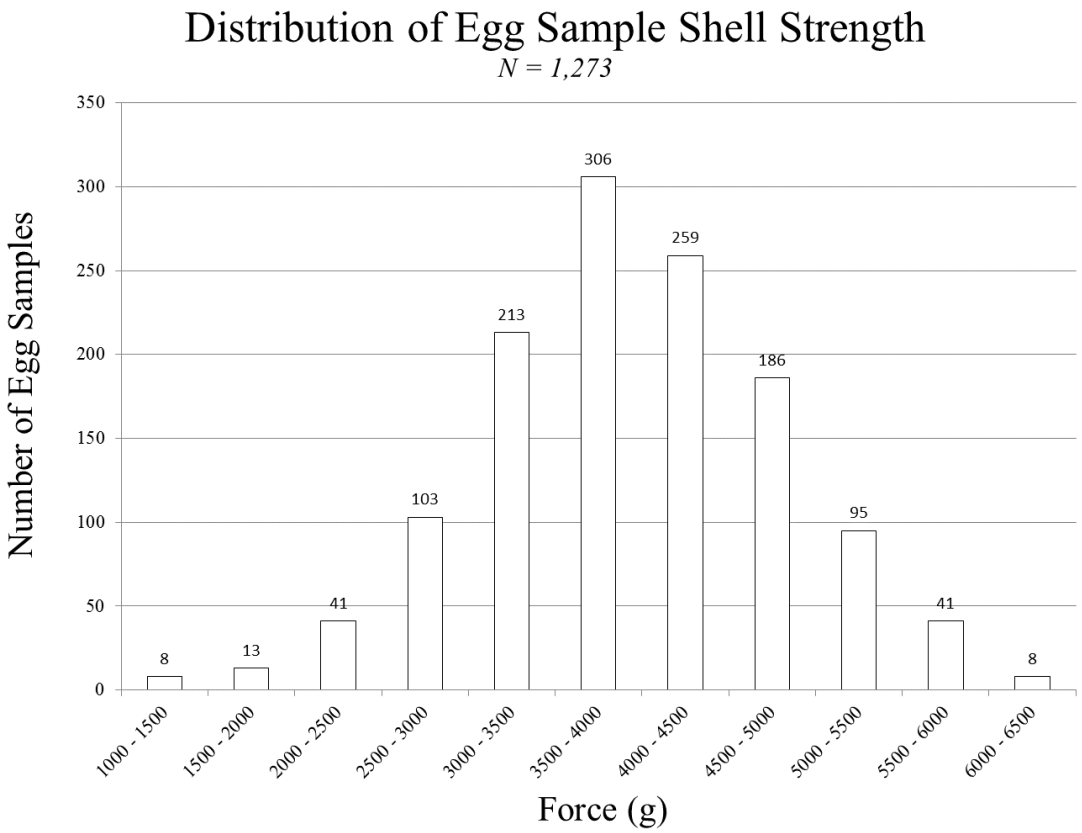

Figure 5.19: Shell strength frequency distribution histogram of non-commercial shell eggs.

N=1,273.

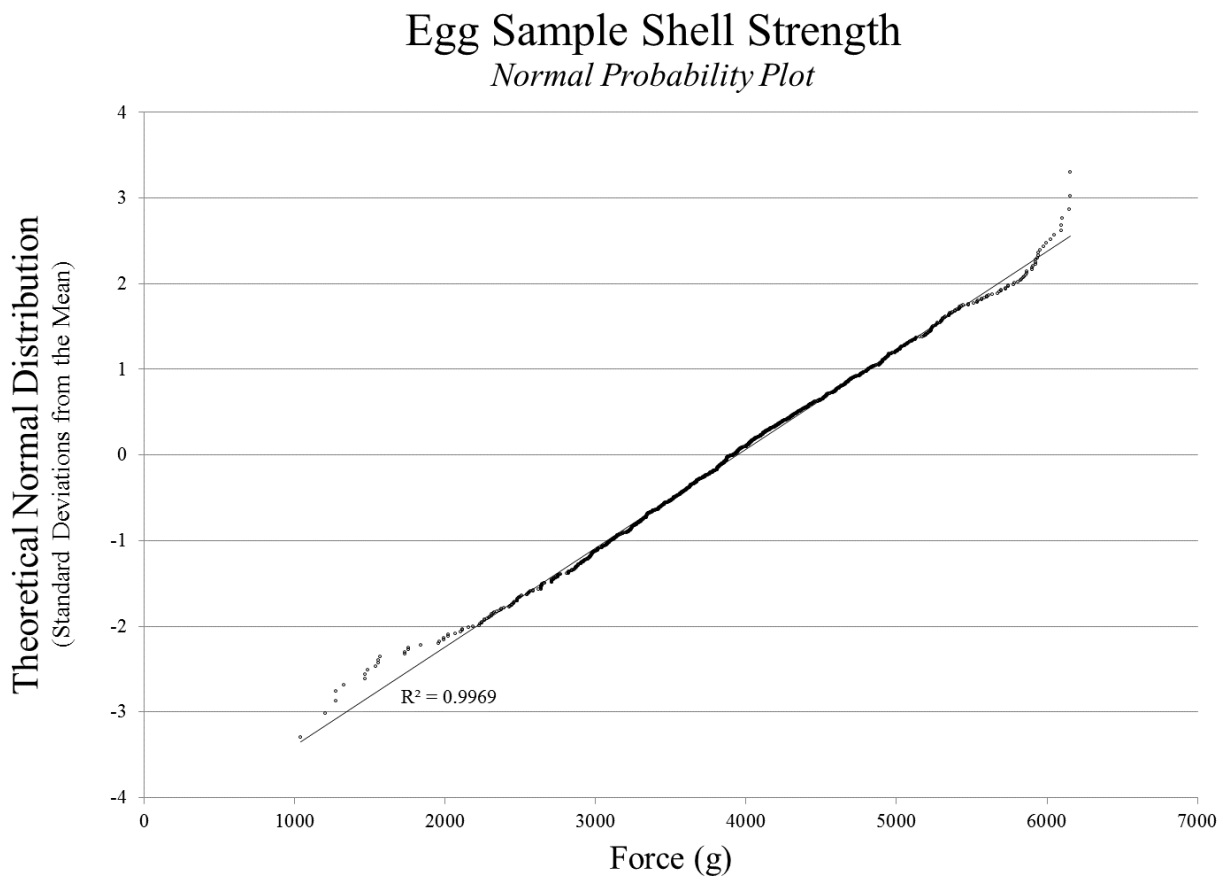

Figure 5.20: Shell strength normality probability plot of non-commercial shell eggs.

n=1,273; R^2=0.9969.

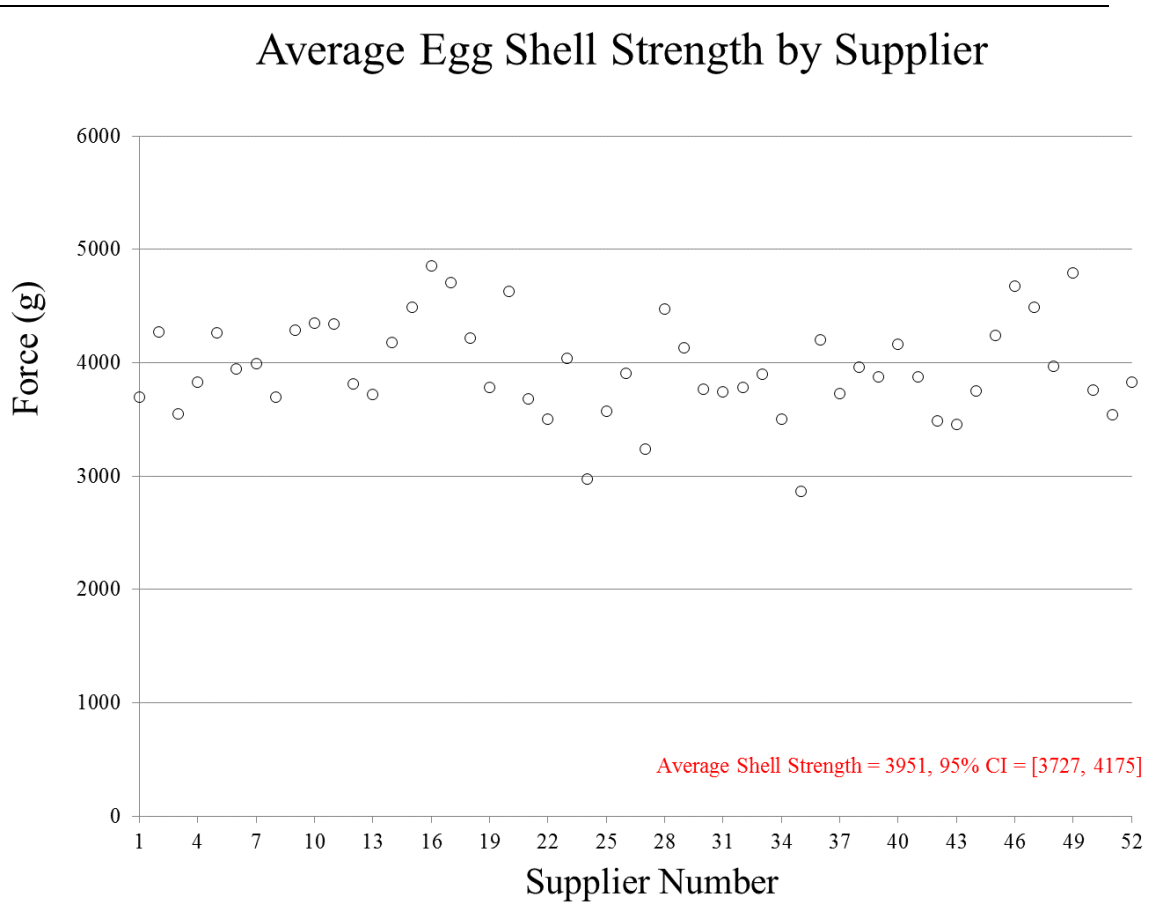

Figure 5.21: Shell strength scatter plot of non-commercial shell eggs by supplier. n=52. Average shell strength of 3,951 g Force with a 95% Confidence Interval (CI) = [3,727, 4,175] g Force.

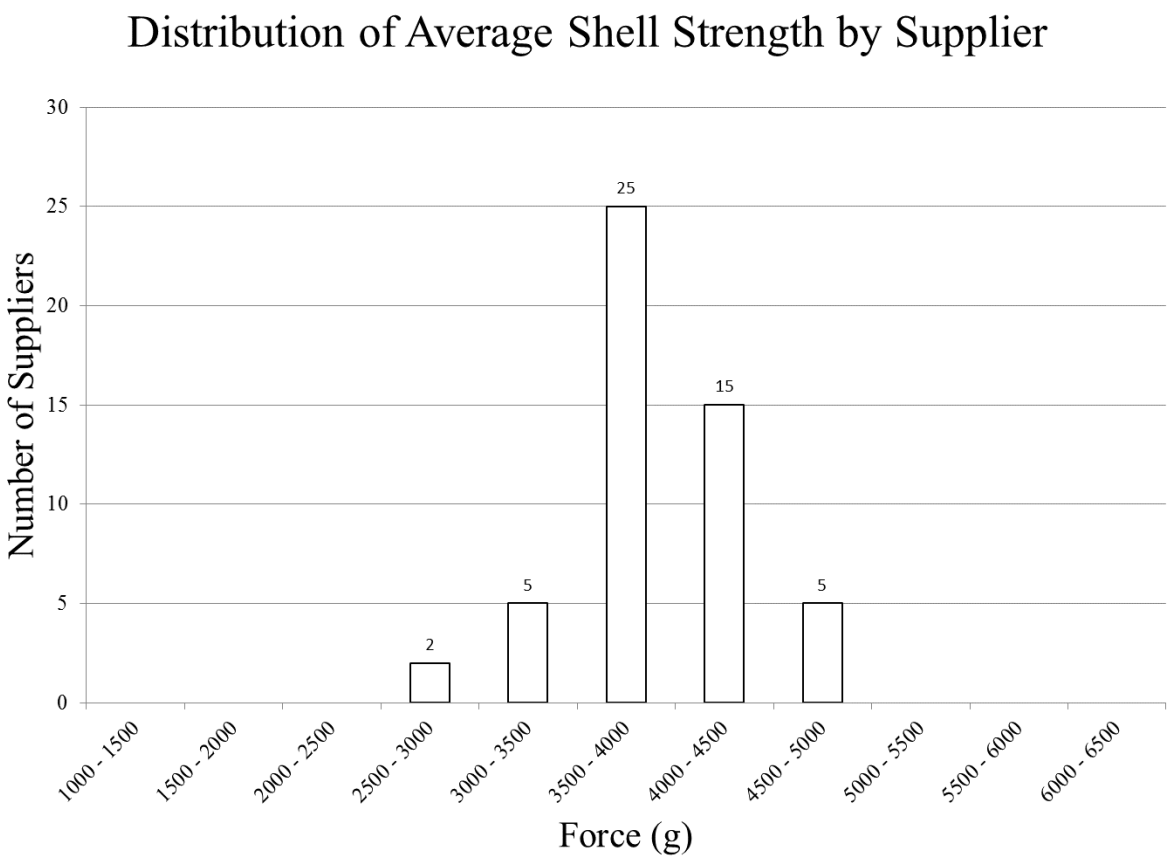

Figure 5.22: Shell strength frequency distribution histogram of non-commercial shell eggs by supplier. n=52

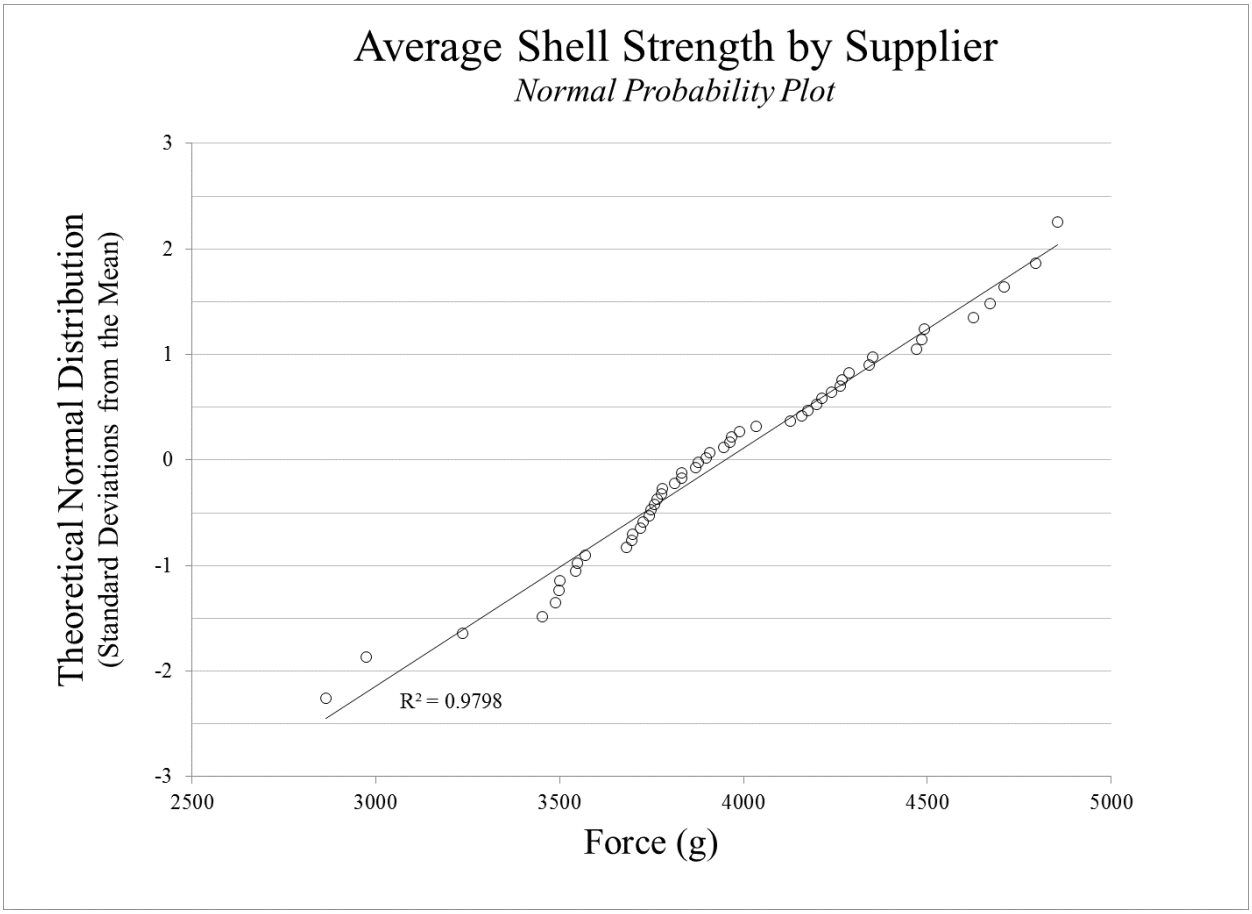

Figure 5.23: Shell strength normality probability plot of non-commercial shell eggs by supplier. n=52; R^2=0.9798.

5.4.4 Vitelline Membrane Strength (VMS)

Overall vitelline membrane strength data (n=1,191) (Figure 5.24) indicates an average VMS of 122.79g Force with a 95% CI = [118.31, 127.27] g Force. Comparative to average VMS of commercially evaluated shell eggs by Jones and others (2018), these samples are lower than in VMS values than those which were: washed; washed, oiled; and unwashed shell eggs and held for a 6 week storage period. After a storage, observed average vitelline membrane strengths for washed (4 ºC); washed, oiled (4 ºC); unwashed (4 ºC); and unwashed (22 ºC); were 155.7, 151.7, 155.0, and 129.9 g Force, respectively (Jones and others, 2018). This comparative average VMS suggests that the sampled non-commercially sourced shell eggs, from this study, may have potentially been stored for up to 6 weeks or longer, which is counter intuitive to most customers' perception.

Vitelline membrane strength frequency distribution (Figure 5.25) indicates the majority of samples evaluated included: 138 samples between 50 and 75 g Force; 181 samples between 75 and 100 g Force; 220 samples between 100 and 125 g Force; and 168 samples between 125 and 150 g; and 140 between 150 and 175 g Force. Normality probability plot of VMS, for all samples, shows R^2=0.9931(Figure 5.26).

Vitelline strength membrane data by supplier (n=52) (Figure 5.27), indicates an average VMS of 127.96 g Force with a 95% Confidence Interval (CI) = [108.31, 147.62] g Force. Vitelline membrane strength by supplier frequency distribution (Figure 5.28) indicates the majority of suppliers' average VMS were: 10 between 100 and 125 g Force); 14 between 125 and 150 g Force; and 10 between 150 and 175 g Force. Normality probability plot of VMS, by supplier, shows R^2=0.9517 (Figure 5.29).

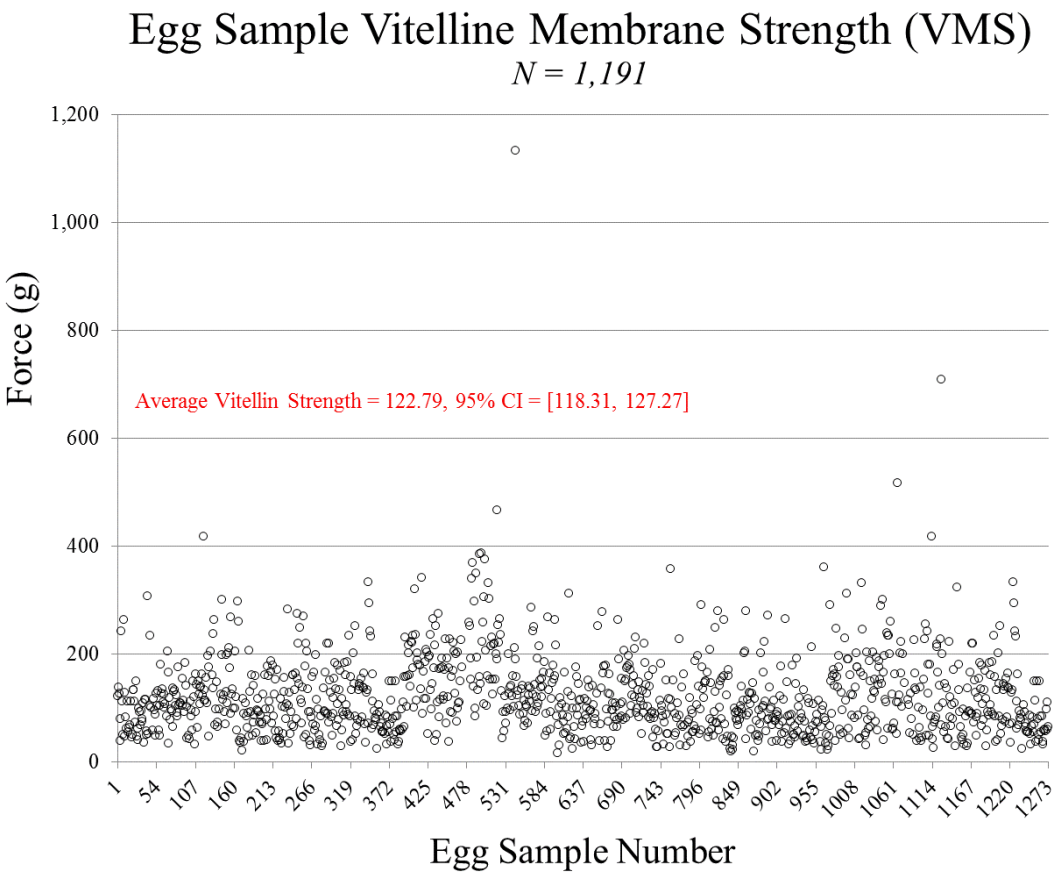

Figure 5.24: Vitelline membrane strength scatter plot of non-commercial shell eggs.

N=1,191. Average vitelline membrane strength of 122.79 g Force with a 95% Confidence Interval (CI) = [118.31, 127.27] g Force.

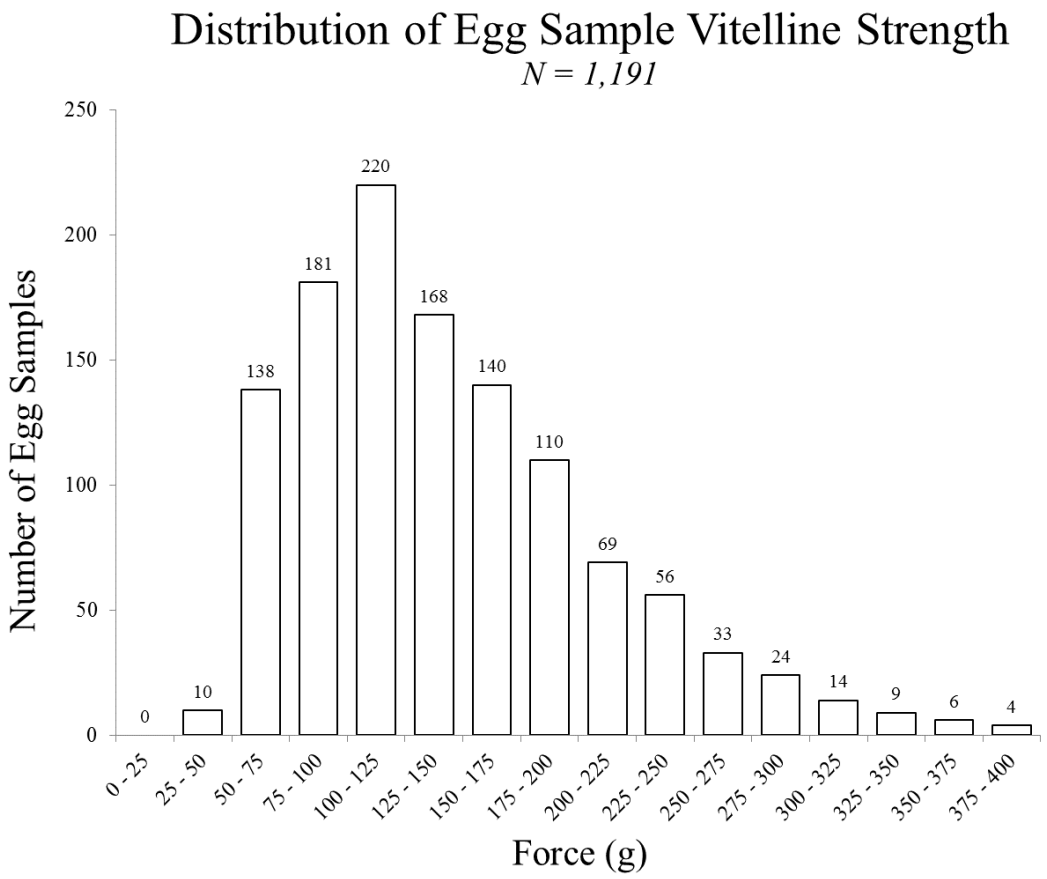

Figure 5.25: Vitelline membrane strength frequency distribution histogram of non-commercial shell eggs. n=1,191.

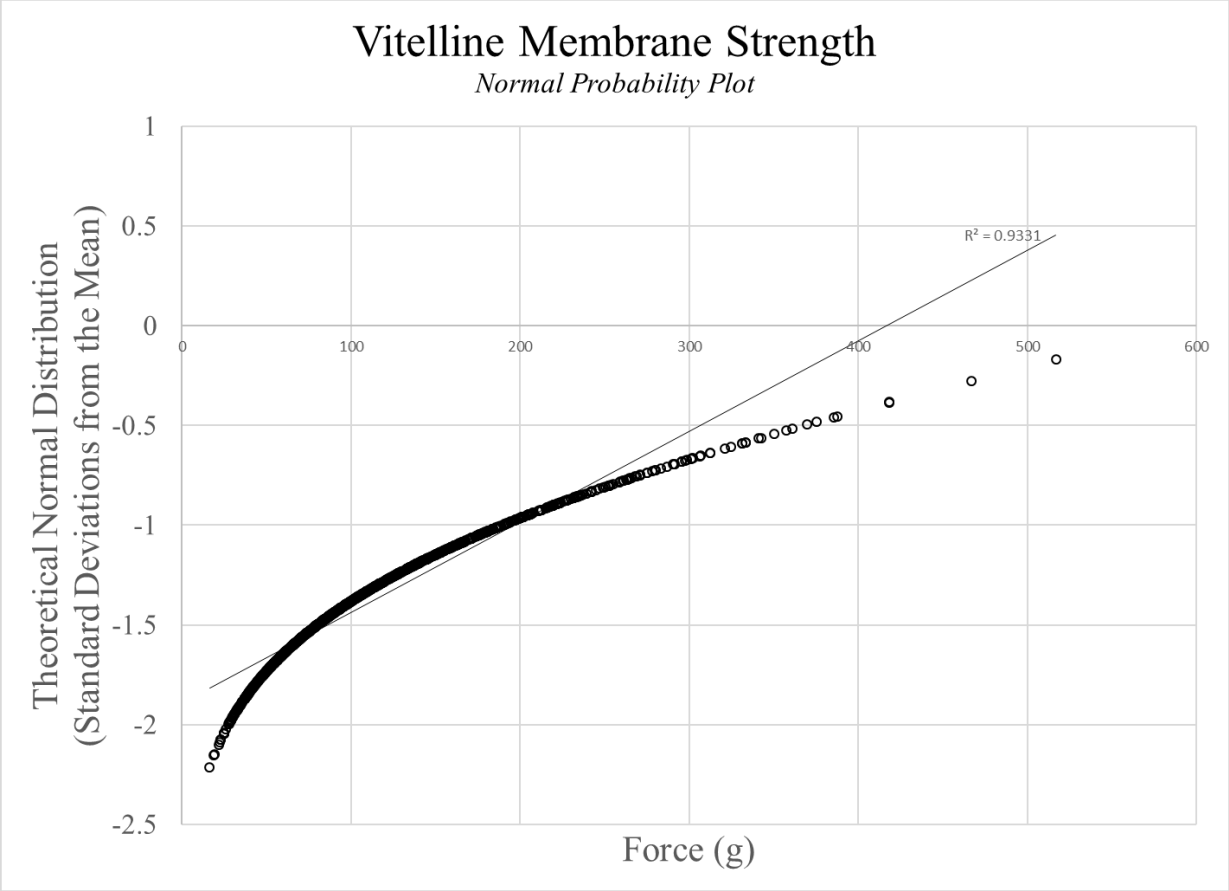

Figure 5.26: Vitelline membrane normality probability plot of non-commercial shell eggs.

n=1,191. R^2=0.9331

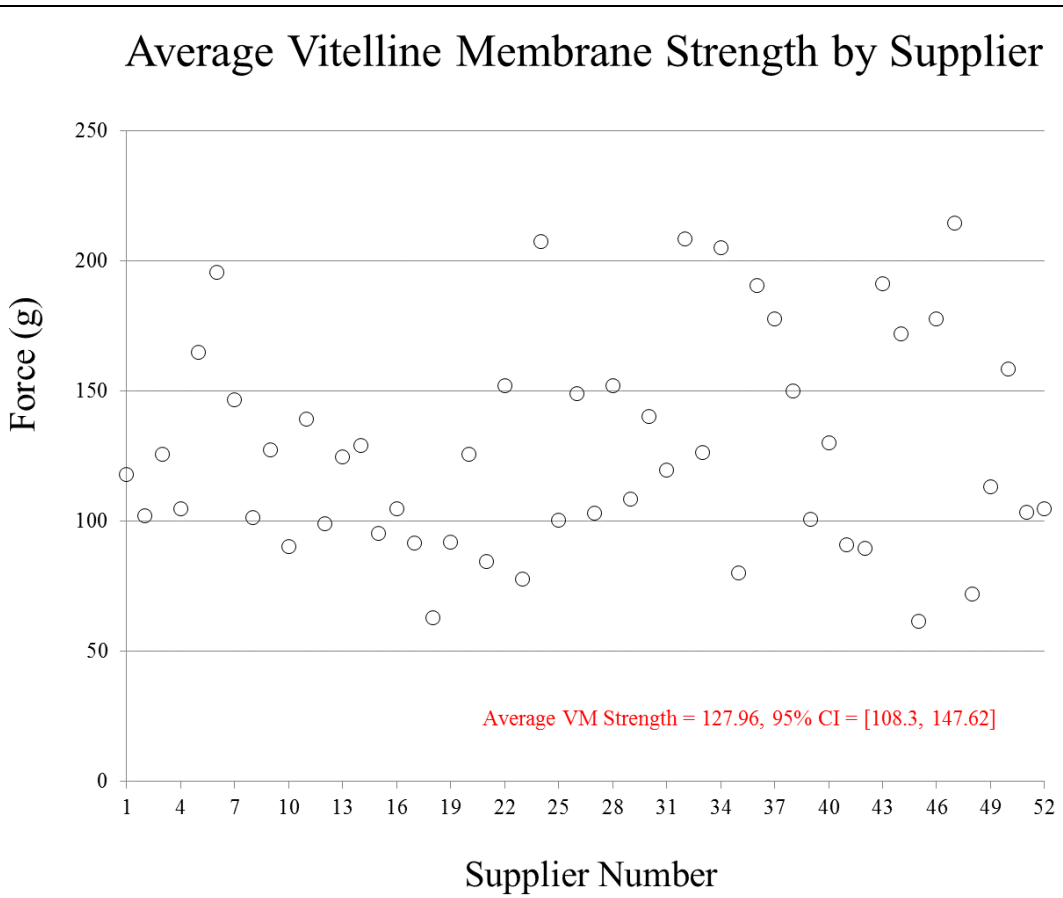

Figure 5.27: Vitelline membrane strength scatter plot of non-commercial shell eggs by supplier. n=51. Average vitelline membrane strength of 127.96 g Force with a 95% Confidence Interval (CI) = [108.31, 147.62] g Force.

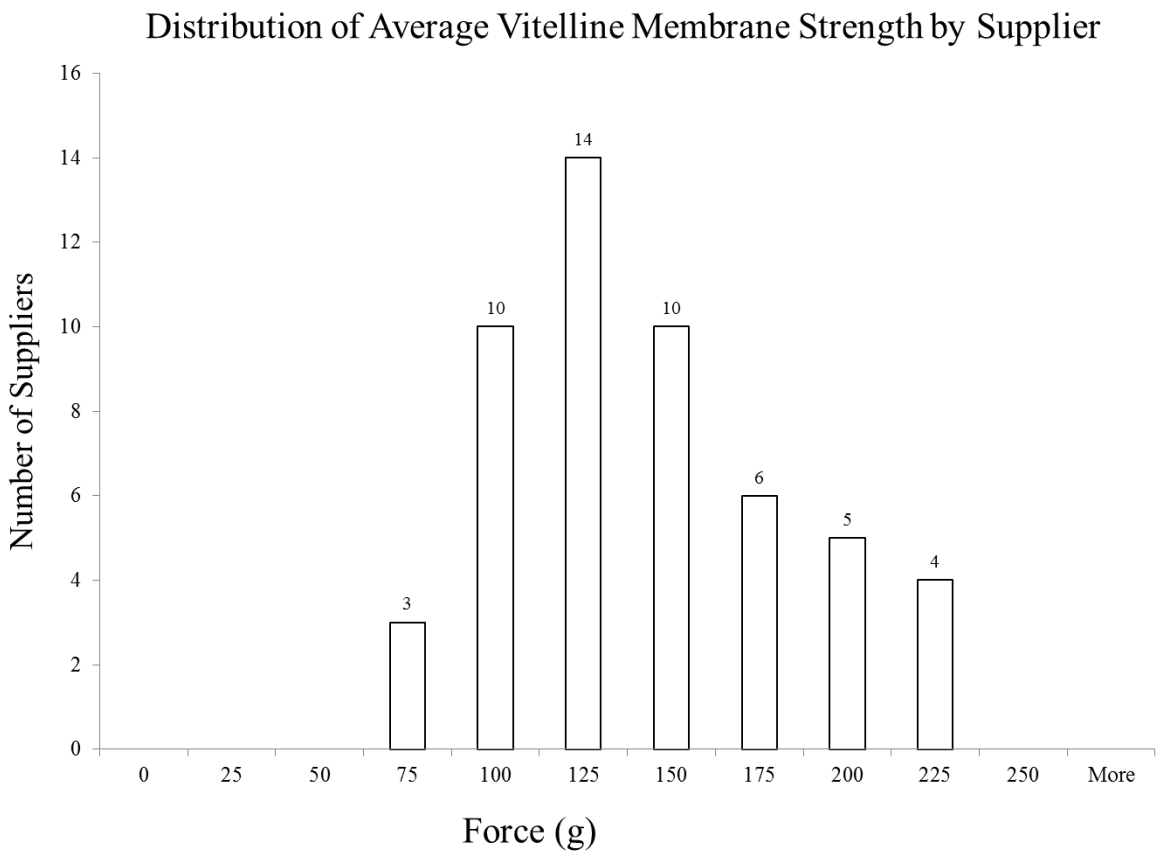

Figure 5.28: Vitelline membrane strength frequency distribution histogram of non-commercial shell eggs by supplier. n=52.

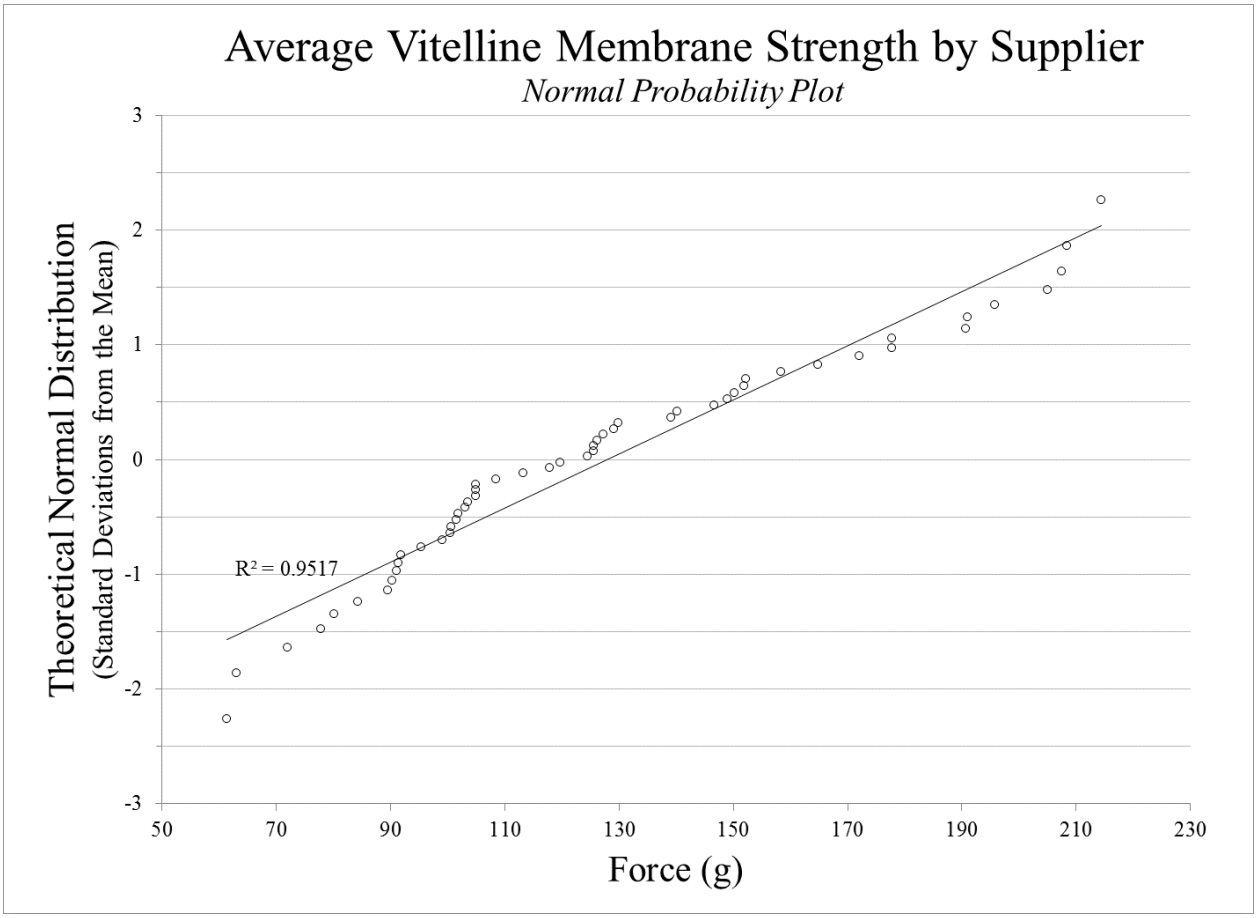

Figure 5.29: Vitelline membrane strength normality probability plot of non-commercial shell eggs by supplier. n=52; R^2=0.9517.

5.6 Conclusions

Based on this study, the quality of shell eggs from non-commercial sources can vary from comparable to retail markets to well below. The data gathered indicates non-commercial shell eggs have a 10-17% frequency of Grade B and a lower vitelline membrane strength; however, comparable shell strength. Additional evaluation with a greater sampling size, may be able to enhance the scope of the data, and possibly provide information on the impact of geography, climate, and overall environmental factors on the quality of non-commercial shell eggs.

CHAPTER VI: SMALL AND LAYER FLOCK SURVEY

6.1 Abstract

Small layer flock (also characterized as non-commercial suppliers) surveys, were collected and evaluated for frequency data on questions targeted to better understand egg handling practices. Of the completed surveys (n=46) flock sizes ranged from 1-10 through 41-60 and flock sources included mainly family/friends/and neighbors, feed stores, internet sites. Layer flock housing included: shed/coop with free-range restrictions during daytime (59%); shed/coop with run (29%); no housing or unrestricted free-range (4%); cages (2%); and other/unspecified (6%). Feed type of non-commercial shell egg suppliers included: mixed rations of purchased feed and kitchen scraps (60%); purchased feed at feed store (38%); and none (2%). In addition, 98% stated nest boxes were provided for their layer flock, and egg collection frequency was reported at: once, daily (67%); twice, daily (27%); every other day (6%). The presence of egg washing within non-commercial shell suppliers was reported at 27%. Egg candling within non-commercial shell egg suppliers were reported at 11%. Egg distribution channels include: neighbors, family, community (73%); farmer's markets (20%); specialty/grocery stores (5%); and restaurants (2%). During storage and transport to distribution, conditions employed by non-commercial suppliers include: room temperature (43%); portable cooler (31%); and refrigeration (26%). Furthermore, packaging of shell eggs by non-commercial shell egg suppliers were reported to consist of recycled egg cartons (85%) and purchased egg cartons (15%). Non-commercial shell egg suppliers are varied in their flock management and egg handling practices. The variances can contribute to the quality and safety of shell eggs, and is important to consider as a consumer to understand the potential impact to the shell eggs being purchased/obtained.

6.2 Introduction

Distribution channels of shell eggs from non-commercial sources (e.g. small and backyard flocks) have expanded over time. Sales have gone beyond personal contacts and roadside stands to include: farmer's markets, health food markets, and farms involved in community-supported agriculture (CSA) programs. Consumer interest in non-commercialized shell eggs are attributed to consumer perceptions of higher safety and quality, relative to their commercially sourced counterparts. Furthermore, the local foods movement also contributes to the rise in consumer demand. Although consumers perceive non-commercial shell eggs with an increased in safety and quality, variable handling, storage, and transportation conditions can contribute to the degradation of the shells eggs prior to reaching the consumer. Thus, the objective of this study was to obtain layer flock management data as a baseline for a greater knowledge on this greatly unknown food source.

6.3 Review of Literature

The popularity of consumer interest in non-commercialized shell eggs is ever growing. Although rural environments tend to be the primary location non-commercialized shell eggs (from small and backyard flocks), live poultry are seen more and more in suburban, as well as urban areas. Historically, small and backyard chickens are privately owned, and generally the resulting products (eggs and meat) are typically not marketed to the general public. Consequently, there is very little information available about these flocks and even less about the eggs they produce**.** Growing distribution channels (e.g. farmer's markets, specialty health food markets, and restaurants) and consumers' demand for greater knowledge of our food supply are the impetus for this study.

A survey, which focused on Los Angeles, Denver, Miami, New York City, found that 1% of households owned chickens (Bahravesh and others, 2014). In addition, 4% planned to become first-time chicken owners within 5 years. In another survey conducted nationwide in the United States (Elkhoraibi and others, 2014), respondents thought that eggs/meat from their chickens tasted better (95%), were safer to consume (84%), and were more nutritious (86%) than store-bought products. In addition, they perceived that the health and welfare of their chickens were better (95%) than on commercial farms.

6.4 Materials and Methods

Convenience random sampling of shell egg suppliers (n=46) competed a small and layer flock survey within the state of Alabama (Figure 6.1). Suppliers of shell eggs, from safety (Chapter 3) and quality evaluations (Chapter 5), included: backyard flocks; health food stores, small farms, and farmers markets.

Figure 6.1: Non-commercial shell egg suppliers sites from small flock layer survey (n=46)

6.5 Results and Discussion

Small layer flock (also characterized as non-commercial suppliers) surveys, were collected and evaluated for frequency data on questions targeted to better understand egg handling practices. Of the collected and completed surveys (n=46), community classification of participants were from rural, suburban, and urban environments at 69%, 20%, and 11%, respectively (Figure 6.2). Non-commercial shell egg suppliers' flock sizes ranged from 1-10 through 41-60 (Figure 6.3). In addition, Figure 6.3 illustrates non-commercial shell egg suppliers comprising of: 52% with a flock size of 11-20; 28% with a flock size of 1-10; 15% with a flock size of 21-40; and 5% with a flock size of 41-60. Flock sources (Figure 6.4) were from family/friends/and neighbors (34%); feed stores (36%); internet sites (6%); and non-specified other sources (24%). Participants indicated the presence of other animals in proximity to their layer flocks at 87% (Figure 6.5). Other animals included: goats, horses, cats, dogs, and geese. Agricultural products produced in proximity of layer flocks of non-commercial egg suppliers included: produce (85%) and other (excluding dairy products OR row crops) (Figure 6.6).

Additionally, care, feed, and housing information on flocks of non-commercial shell egg suppliers was gathered. Layer flock housing of non-commercial egg suppliers (Figure 6.7) included: shed/coop with free-range restrictions during daytime (59%); shed/coop with run (29%); no housing or unrestricted free-range (4%); cages (2%); and other/unspecified (6%). Feed type (Figure 6.8) choice of non-commercial shell egg suppliers included: mixed rations of purchased feed and kitchen scraps (60%); purchased feed at feed store (38%); and none (2%). In addition, non-commercial shell egg suppliers provides calcium supplements at frequencies of: occasionally (70%); daily (11%); weekly (6%), and never (13%) (Figure 6.9). Medications

provided to layer flocks (over the past 12 months) of non-commercial shell egg suppliers included: Coccidiosis preventative (27%); deworming (18%); antibiotics (10%); other (5%); and none (40%)(Figure 6.10). Furthermore, veterinary services provided to layer flocks of non-commercial shell egg supplier were stated at 11% (Figure 6.11).

Of all non-commercial shell egg suppliers whom participated, 98% stated nest boxes were provided for their layer flock (Figure 6.12). Nest boxes allow for ease in locating eggs and which promotes frequency in collection. Frequent egg collection is critical in preserving the quality of shell eggs. Egg collection frequency of non-commercial shell egg suppliers were reported at: once, daily (67%); twice, daily (27%); every other day (6%) (Figure 6.13). The presence of egg washing within non-commercial shell suppliers was reported at 27% (Figure 6.14). Washing techniques varied from: dry wiping; rinsing with cold running water, cleaning with detergents, sanitizing with a bleach solution, and/or a combination. Egg candling within non-commercial shell egg suppliers were reported at 11% (Figure 6.15). Candling allows for grading of eggs, as a non-destructive method. In this study, 89% of non-commercial shell egg suppliers do not candle, possibly due to time/labor constraints or availability of a candling apparatus. Nonetheless, lack of candling allows for ambiguity to the quality of eggs provided from these suppliers.

Egg distribution channels of non-commercial shell egg suppliers are indicated in Figure 6.16 and include: neighbors, family, community (73%); farmer's markets (20%); specialty/grocery stores (5%); and restaurants (2%). During storage and transport to distribution of shell eggs, conditions employed by non-commercial suppliers include: room temperature (43%); portable cooler (31%); and refrigeration (26%) (Figure 6.17). The 43% of non-commercial shell egg suppliers is important to highlight, since storing and transporting shell eggs

under refrigerated temperature is critical to maintaining its quality. Finally, packaging of shell eggs by non-commercial shell egg suppliers were reported to consist of recycled egg cartons (85%) and purchased egg cartons (15%) (Figure 6.18). Recycling is a common practice in our consumer driven society, and extremely valuable to a sustainable ecosystem; However, it is equally important to understand that recycled shell egg packaging may harbor potential health hazards (e.g. *Salmonella*), and special care (use of cleanable and sanitizable plastic cartons) may want to be considered.

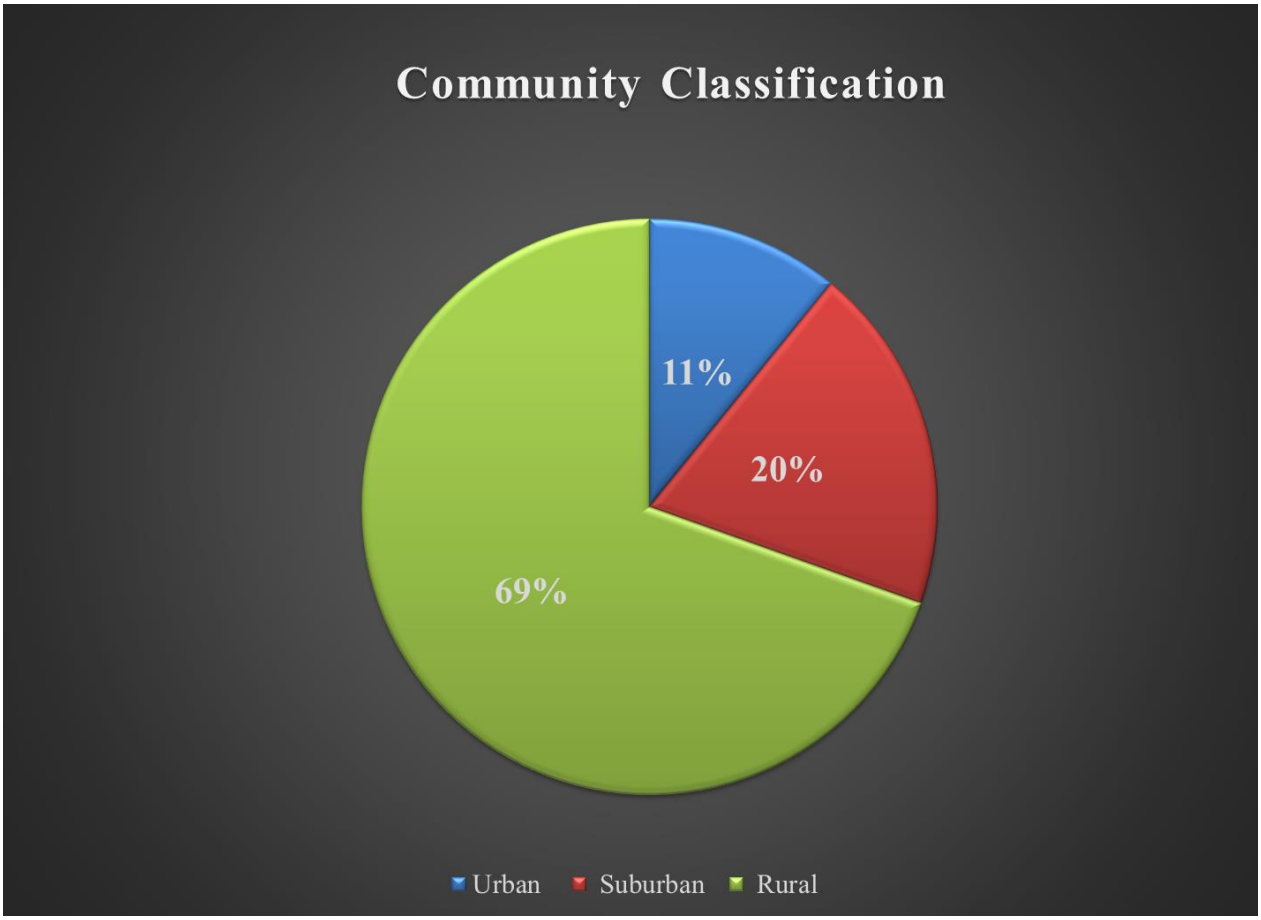

Figure 6.2: Community classification of non-commercial shell egg suppliers (n=46).

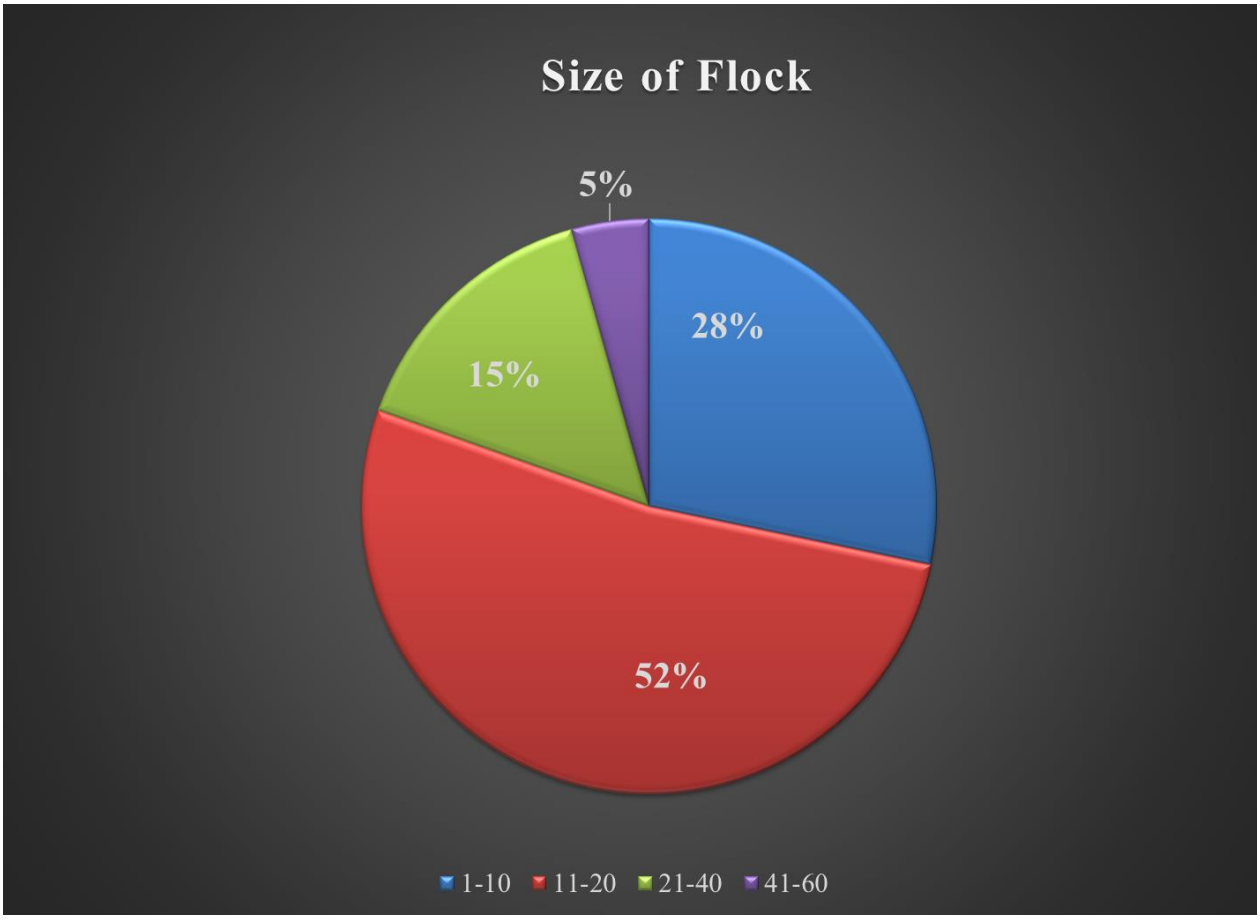

Figure 6.3: Flock size of non-commercial egg suppliers (n=46).

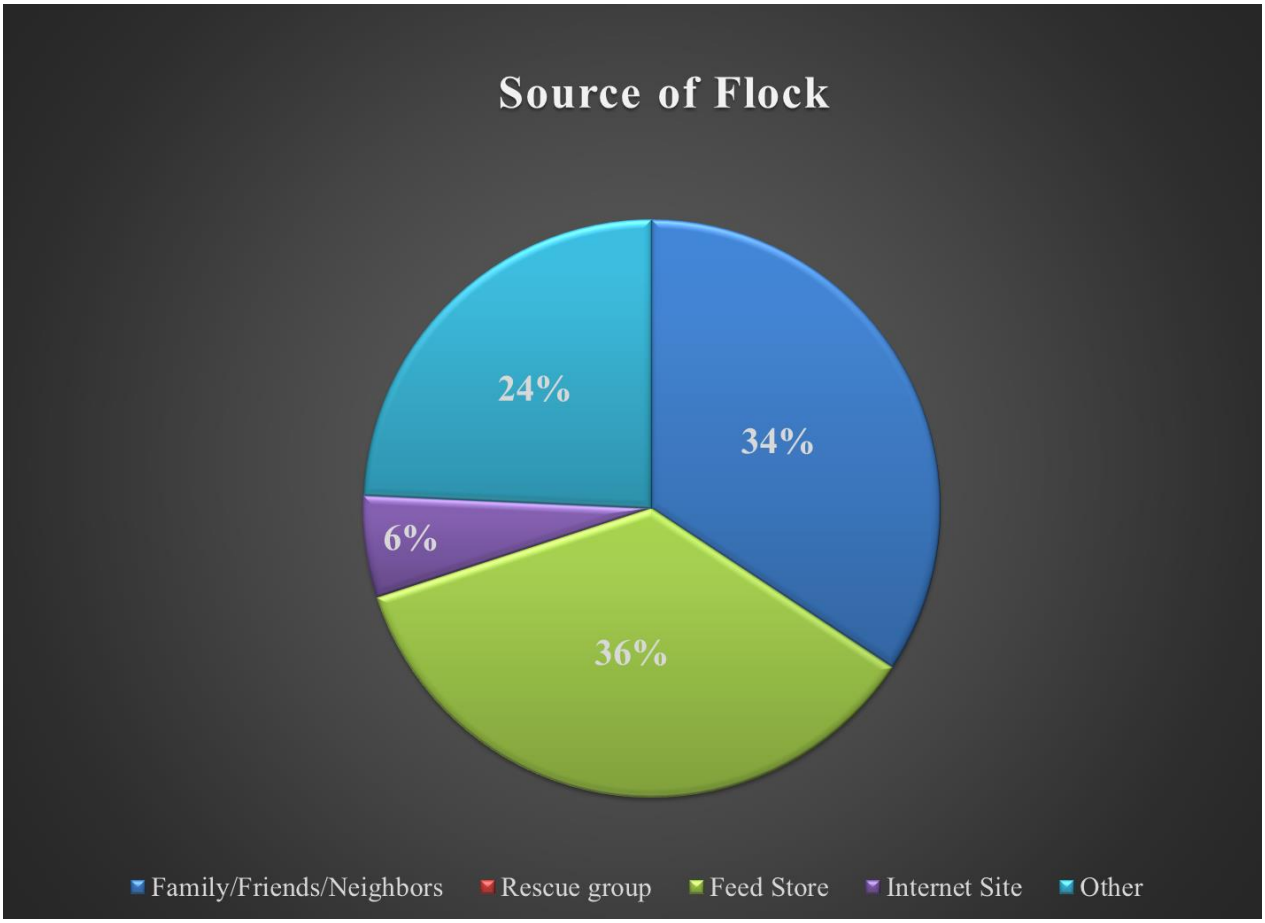

Figure 6.4: Source of layer flock size of non-commercial shell egg suppliers (n=46). Note: Although available as an option response, zero suppliers indicated "Rescue group" as a source of their flock.

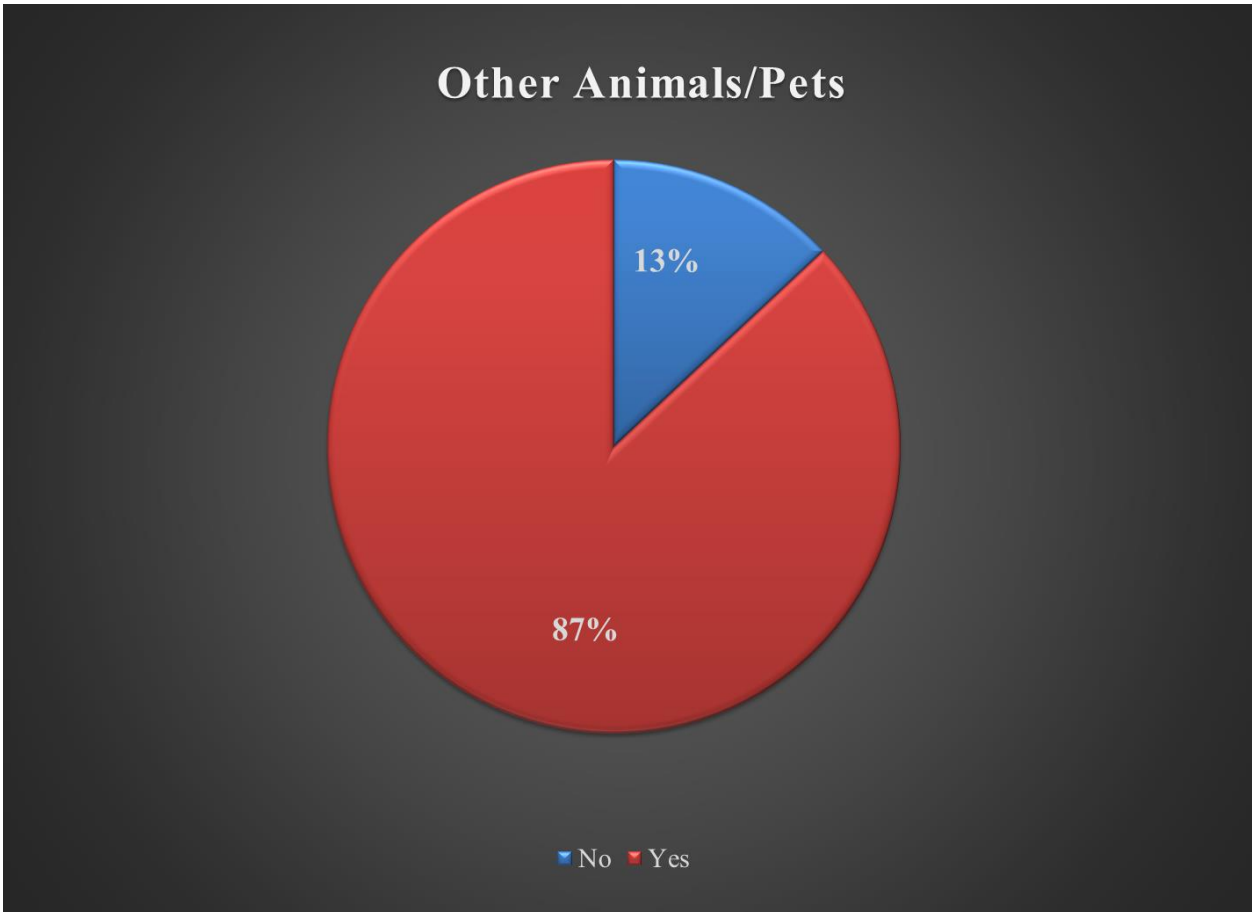

Figure 6.5: Presence of other animals within layer flock of non-commercial shell egg suppliers (n=46).

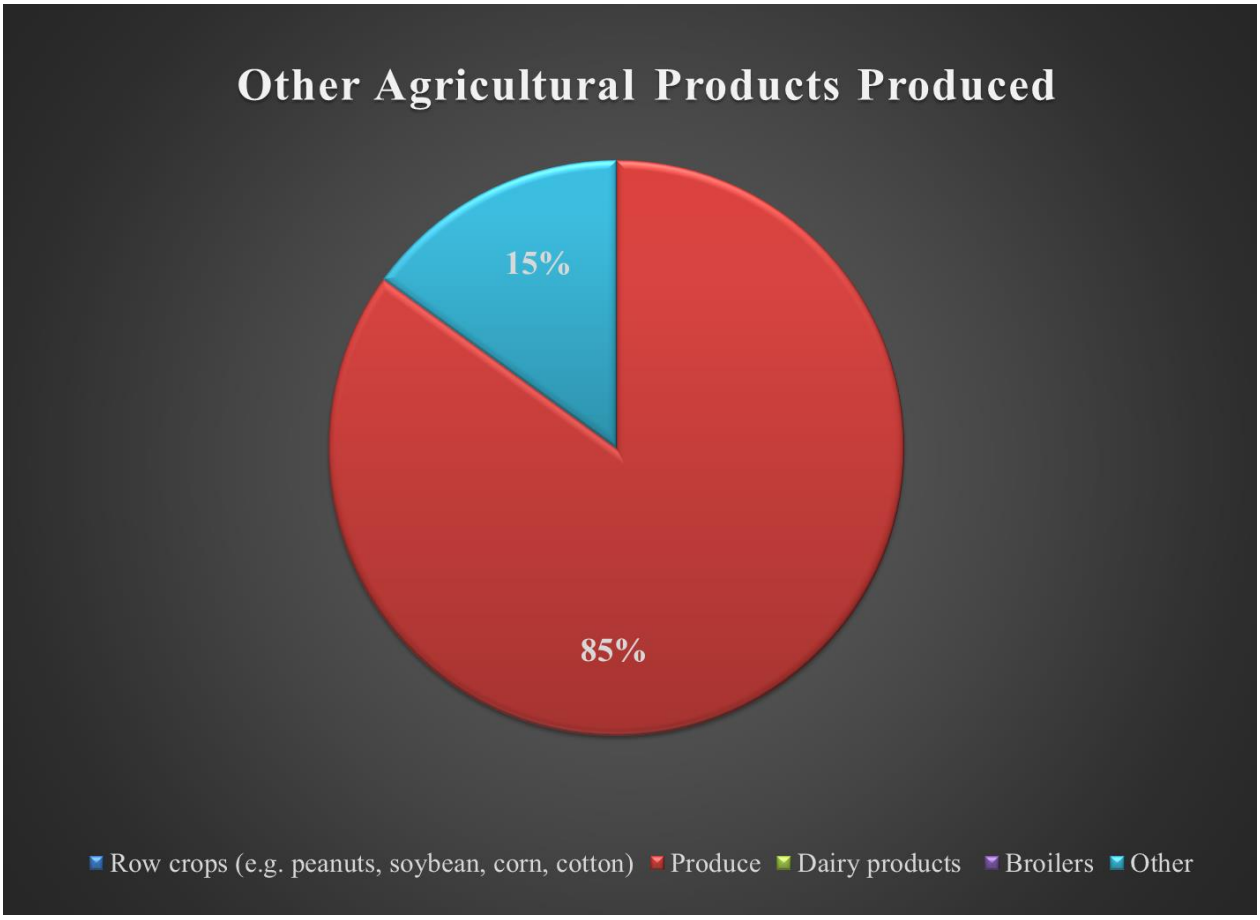

Figure 6.6: Agricultural products produced near layer flock of non-commercial shell egg suppliers (n=46). Note: Although available as an option response, zero suppliers indicated "Dairy products", "Broilers" or "Other" as a response for other agricultural products produced.

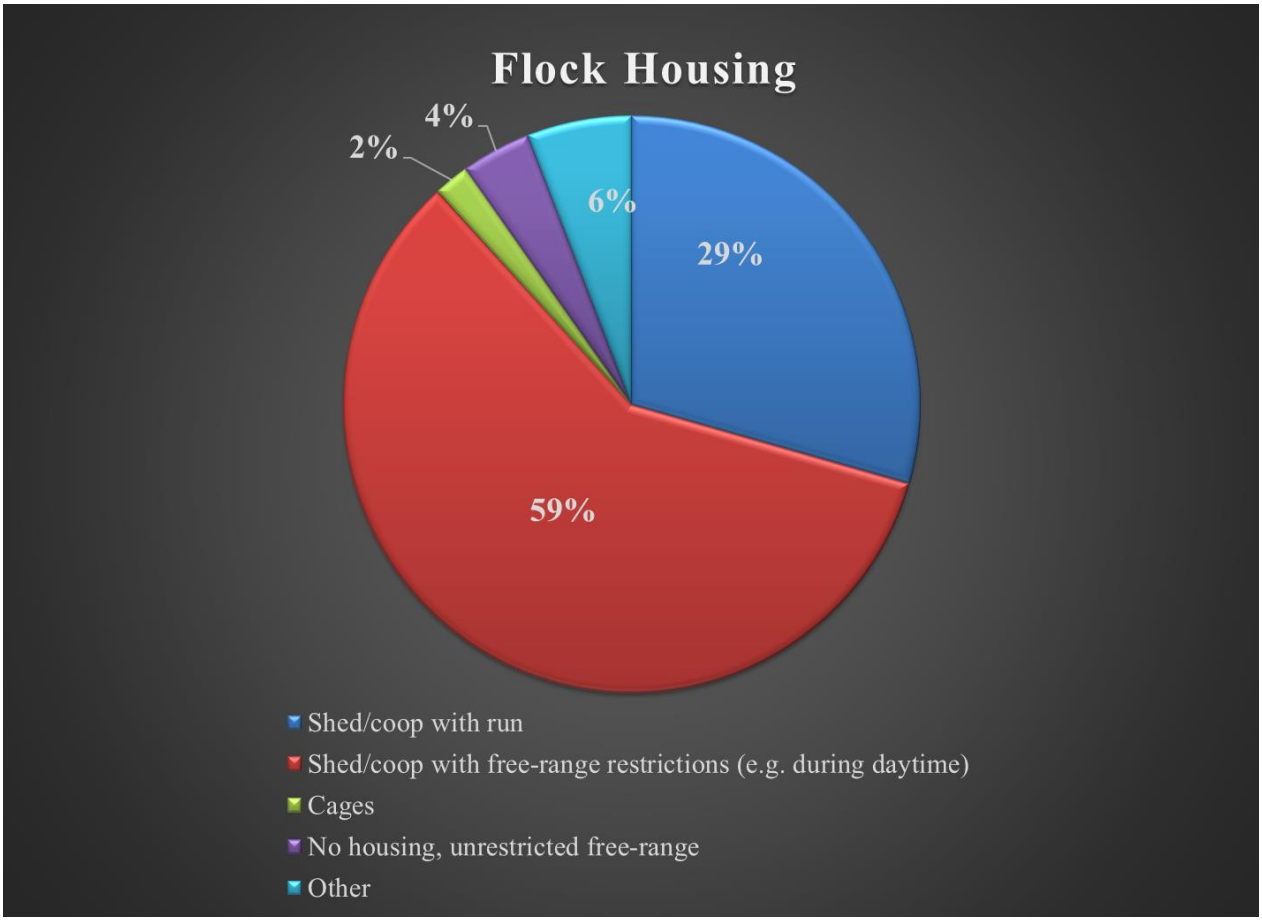

Figure 6.7: Layer flock housing of non-commercial shell egg suppliers (n=46).

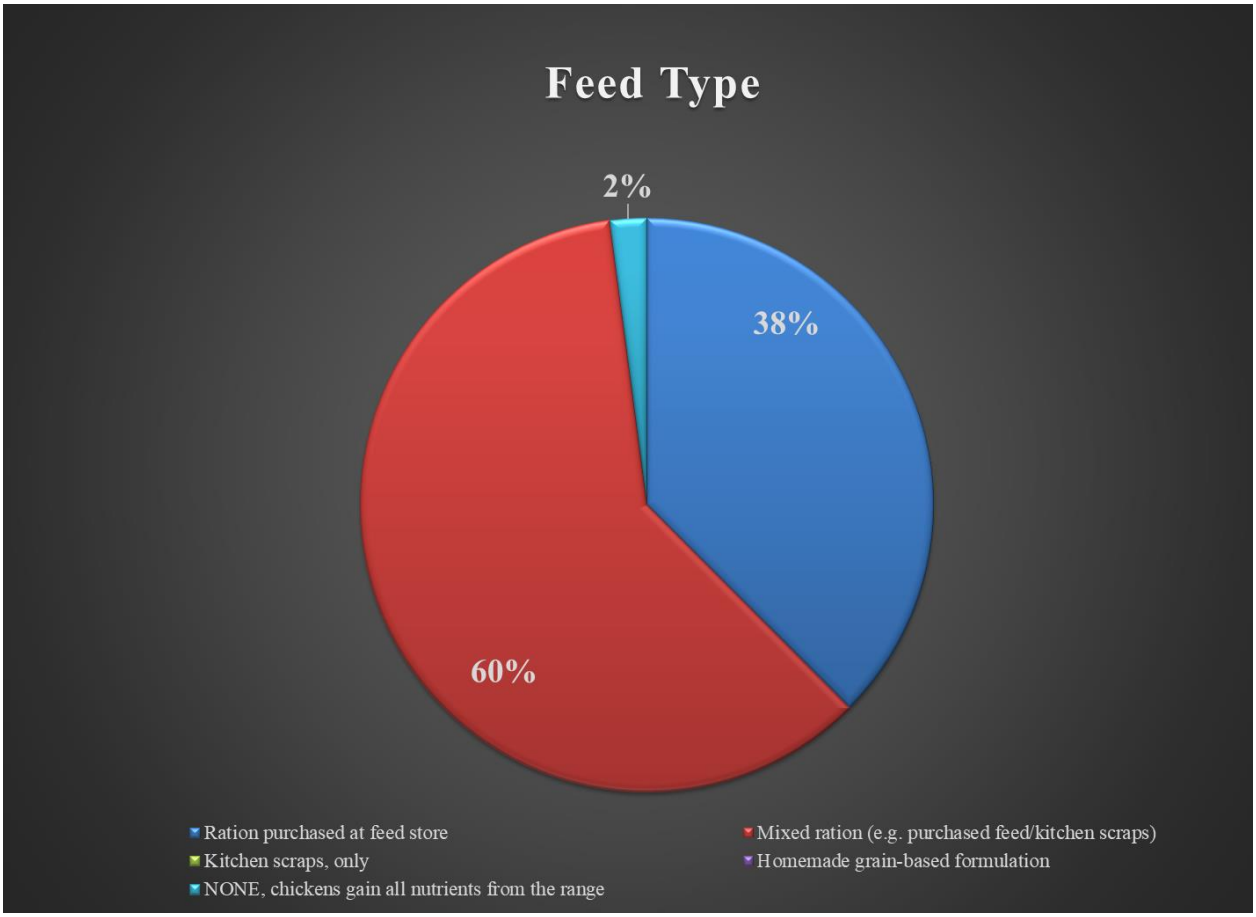

Figure 6.8: Layer flock feed type of non-commercial shell egg suppliers (n=46).

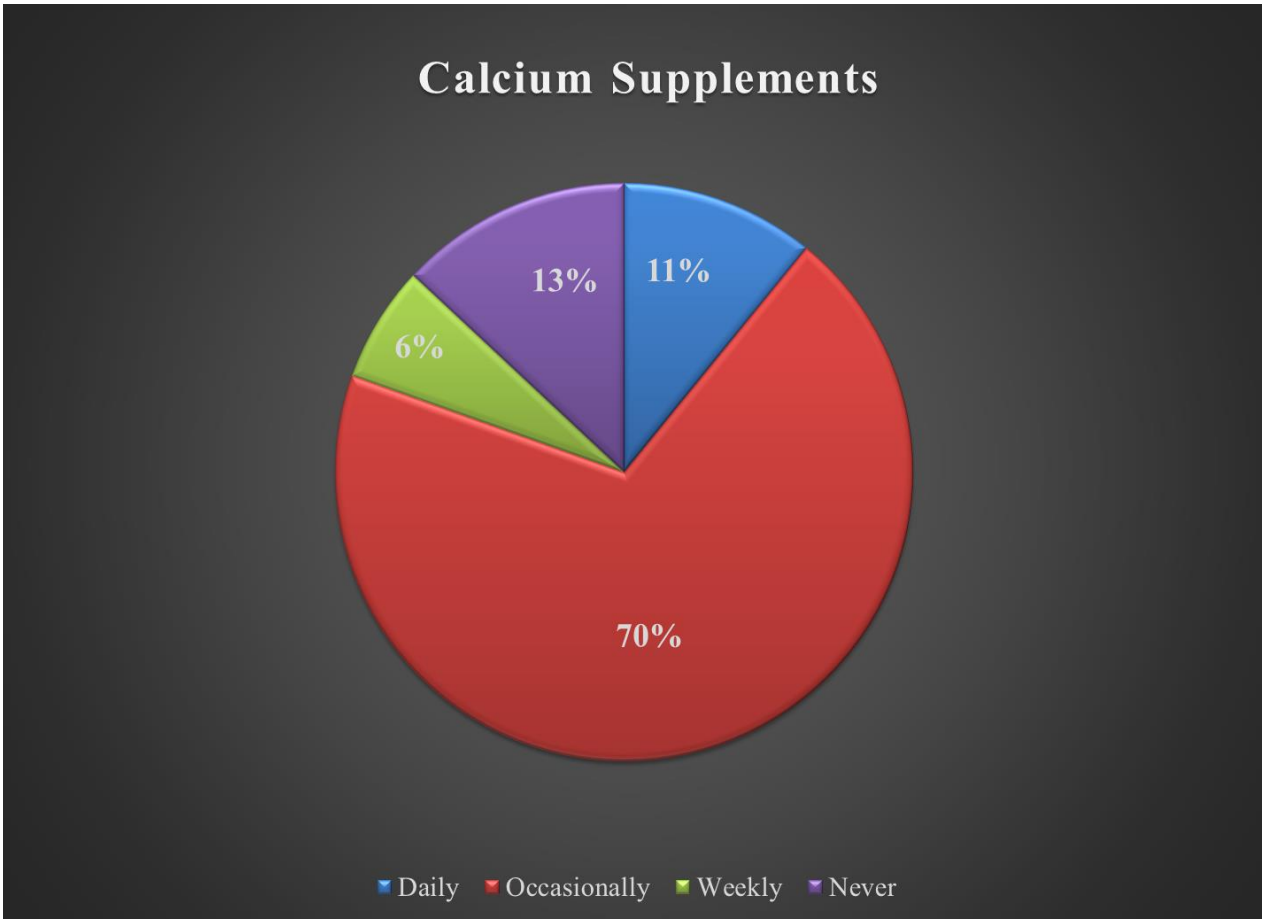

Figure 6.9: Calcium supplement allowance to layer flocks of non-commercial shell egg suppliers (n=46).

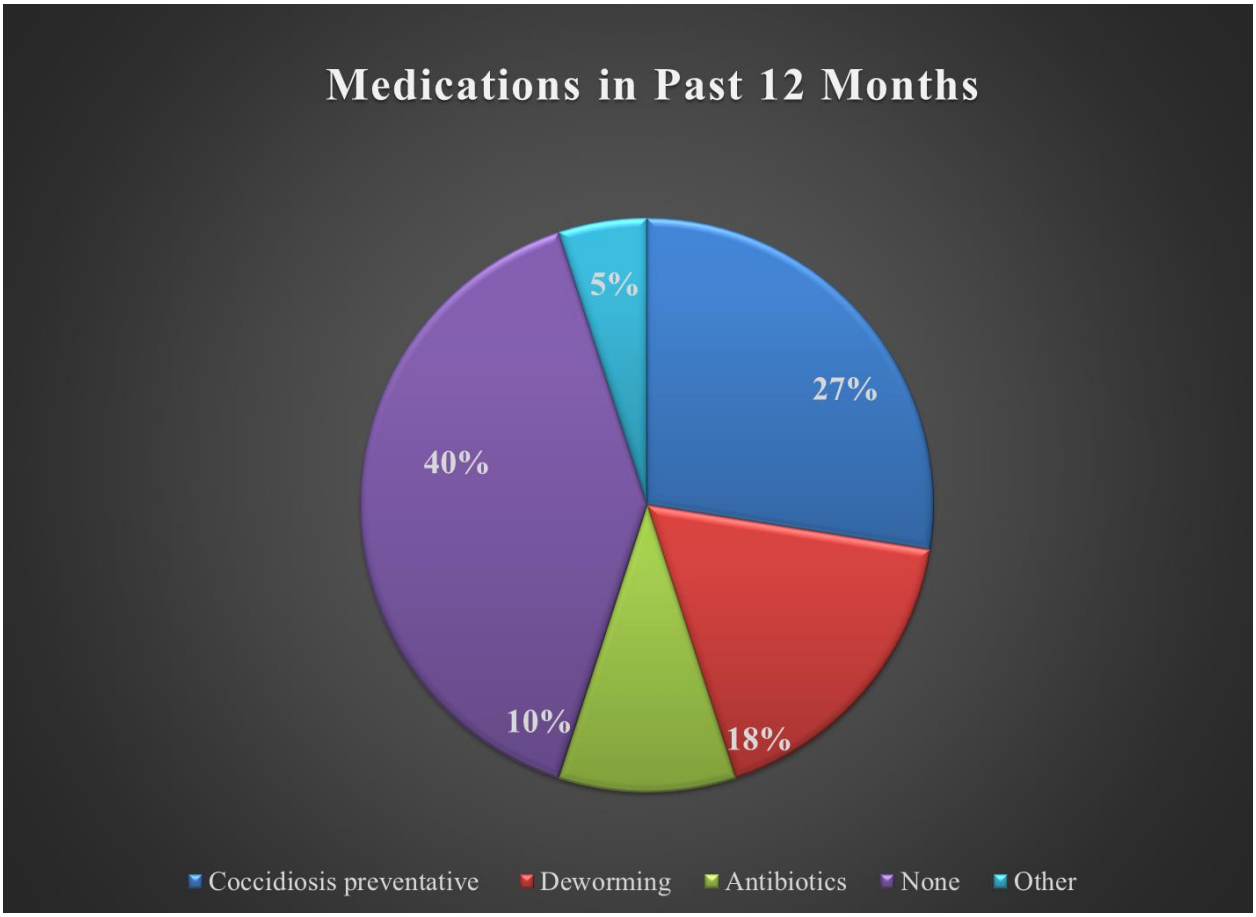

Figure 6.10: Medications provided to layer flock of non-commercial shell egg suppliers (n=46).

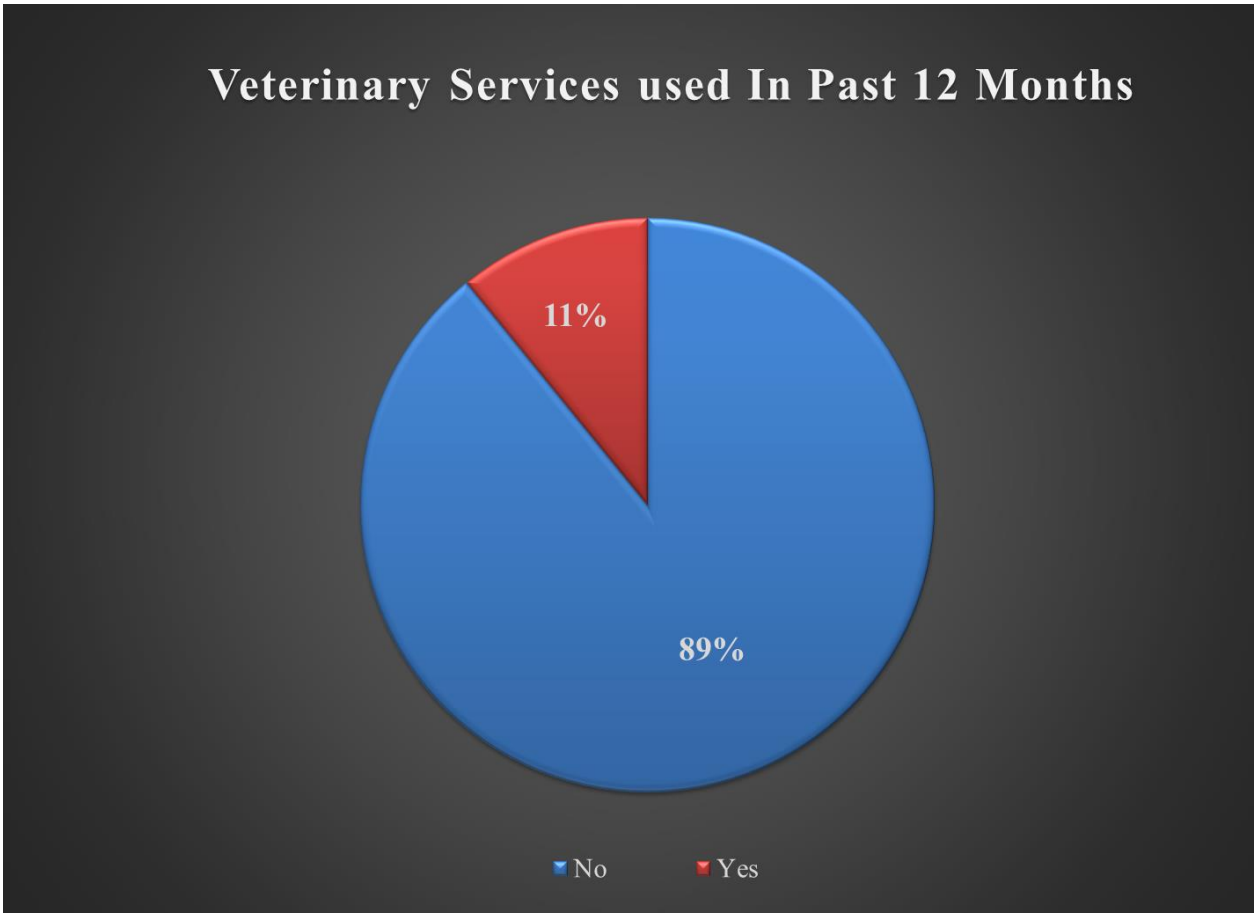

Figure 6.11: Presence of veterinary services to layer flock of non-commercial shell egg suppliers

(n=46).

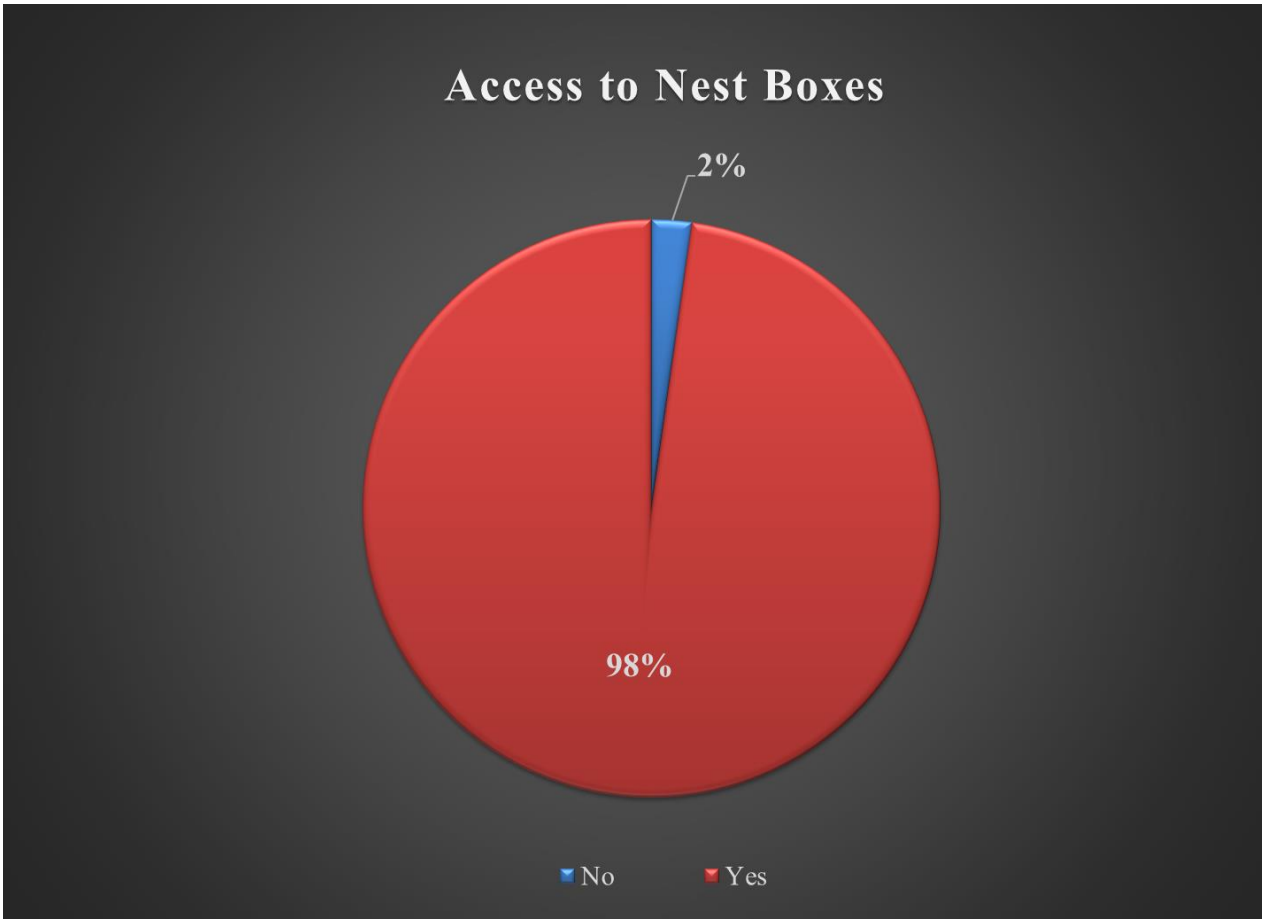

Figure 6.12: Non-commercial shell egg suppliers (n=46) **which provide their layer flocks access to nest boxes.**

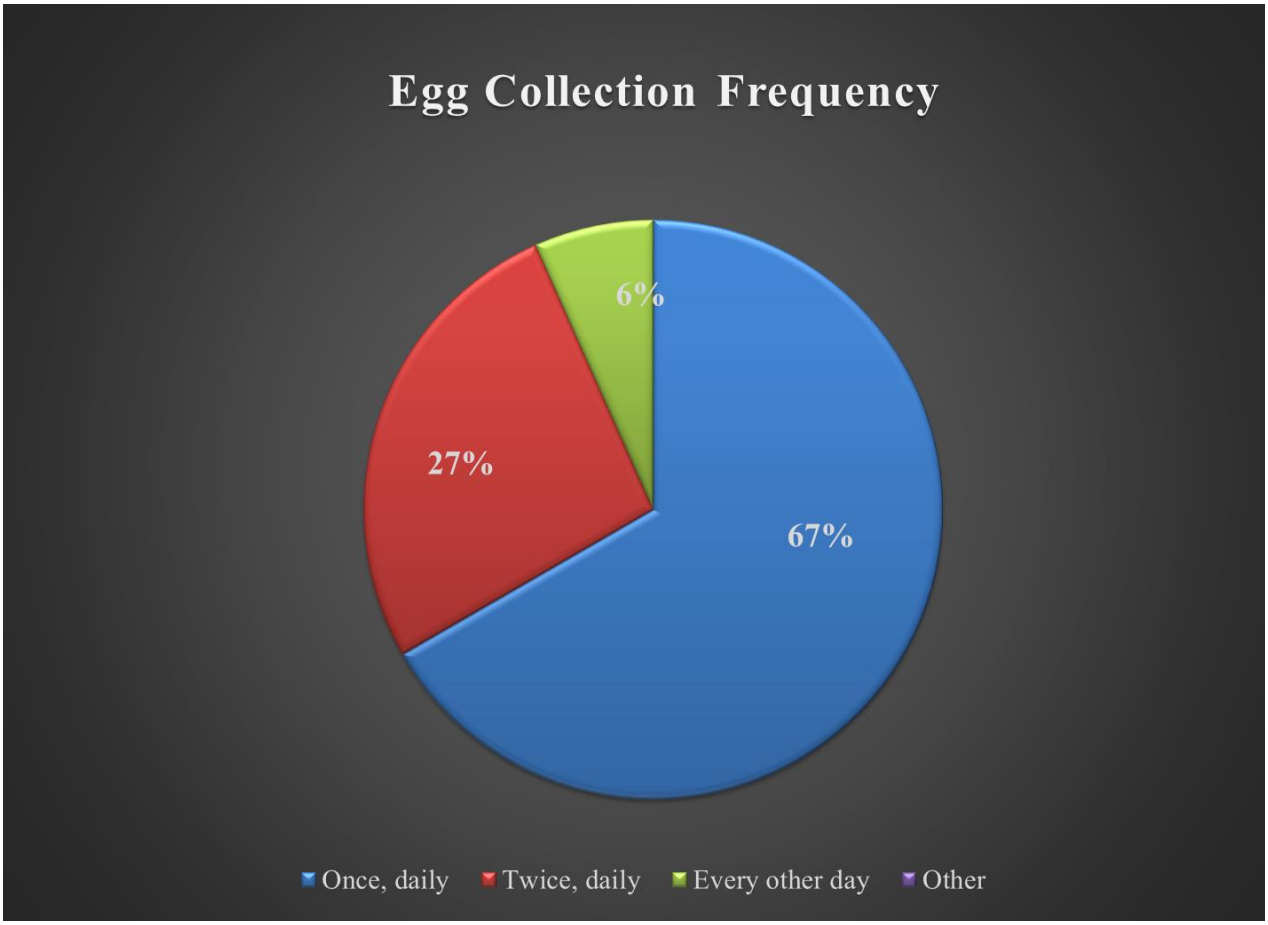

Figure 6.13: Egg collection frequency of non-commercial shell egg suppliers (n=46). Note: Although an option response, zero suppliers indicated "Other" to the egg collection frequency question.

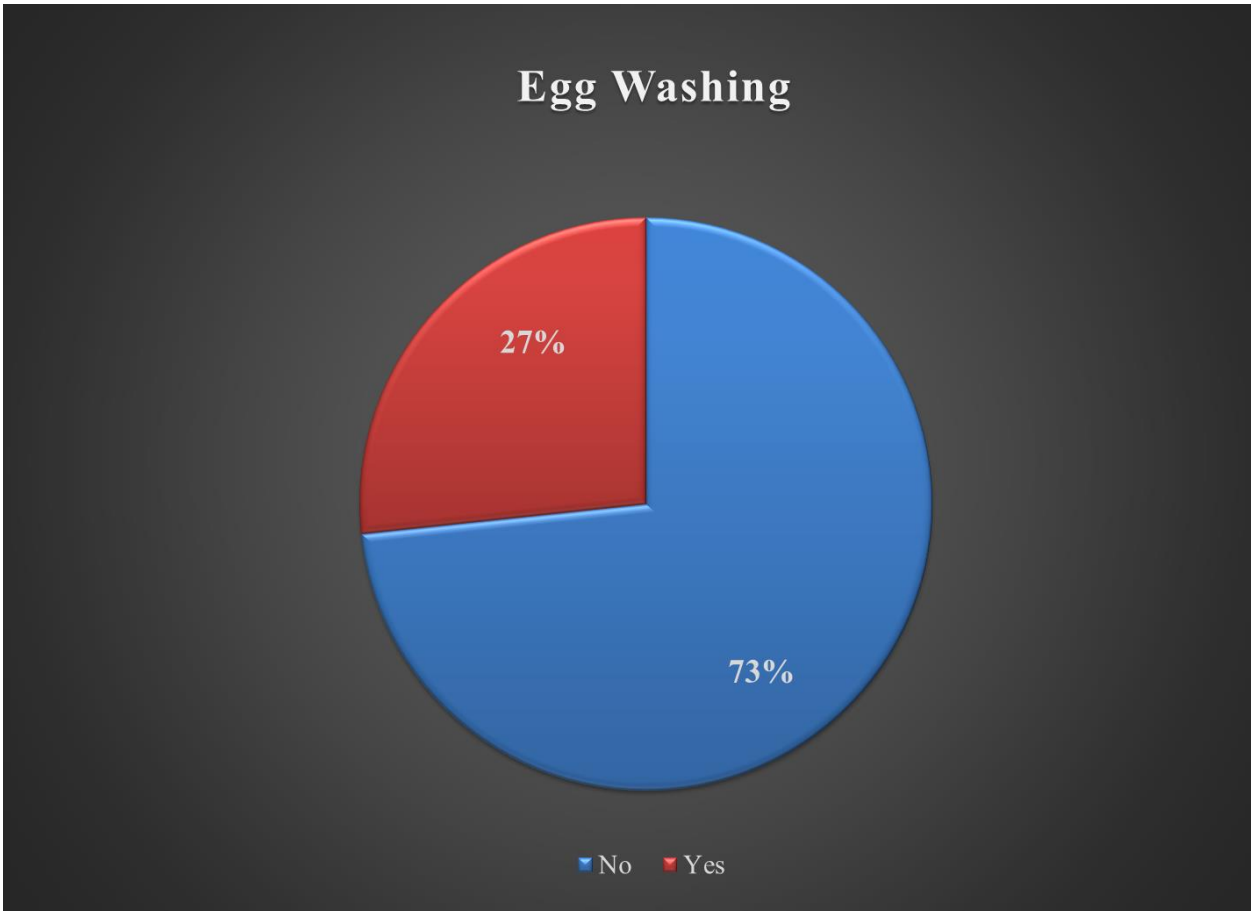

Figure 6.14: Presence of egg washing with non-commercial shell egg suppliers (n=46).

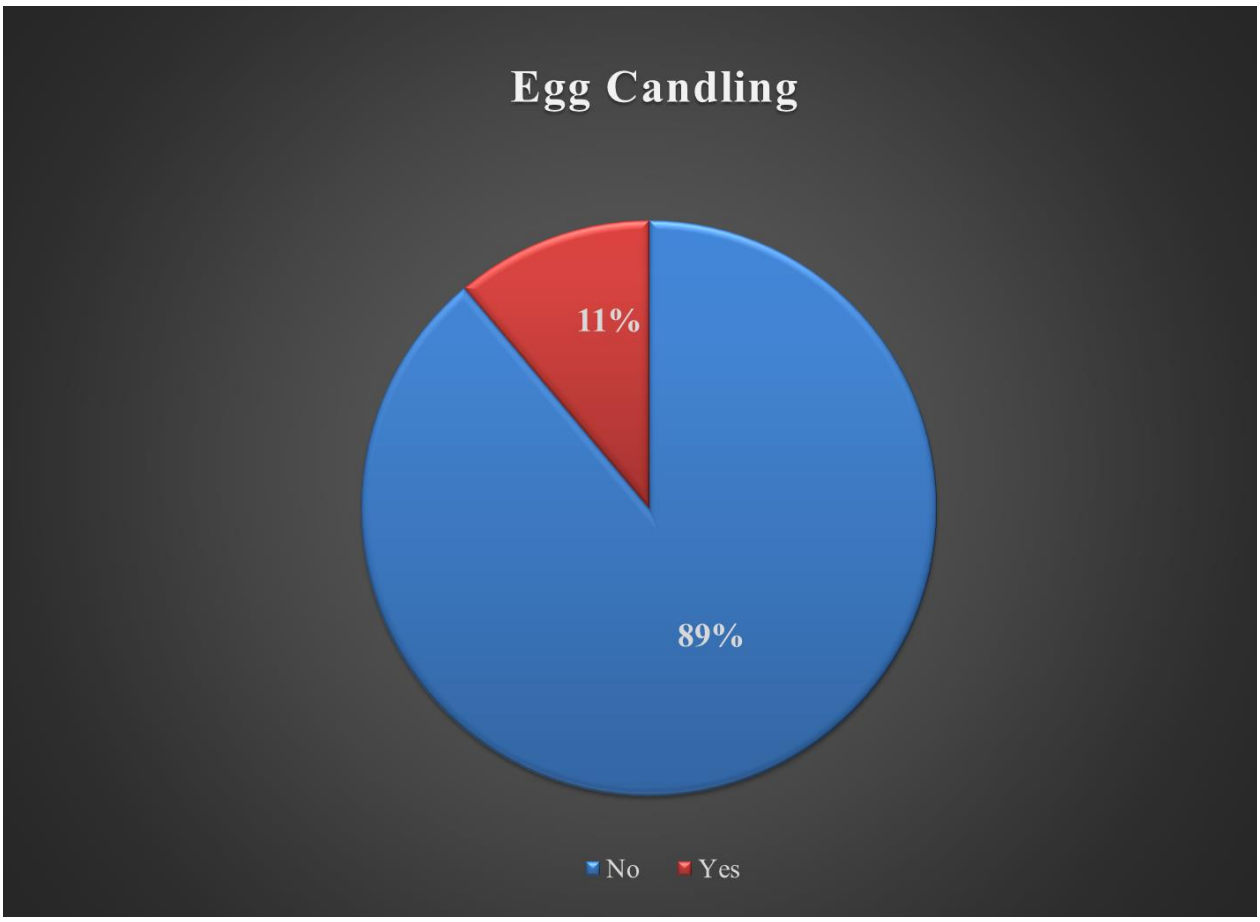

Figure 6.15: Presence of egg candling with non-commercial shell egg suppliers (n=46).

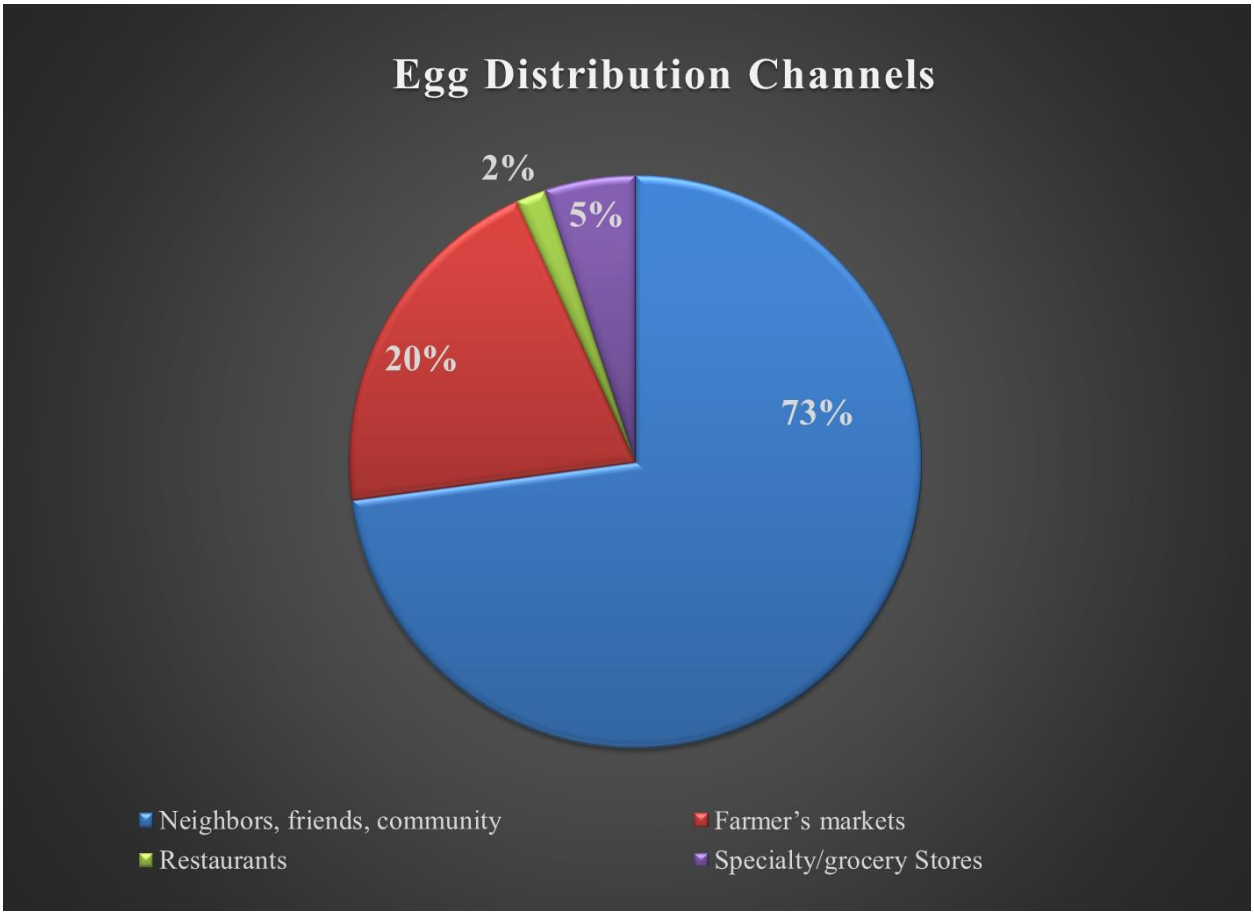

Figure 6.16: Egg distribution channels of non-commercial shell egg suppliers (n=46).

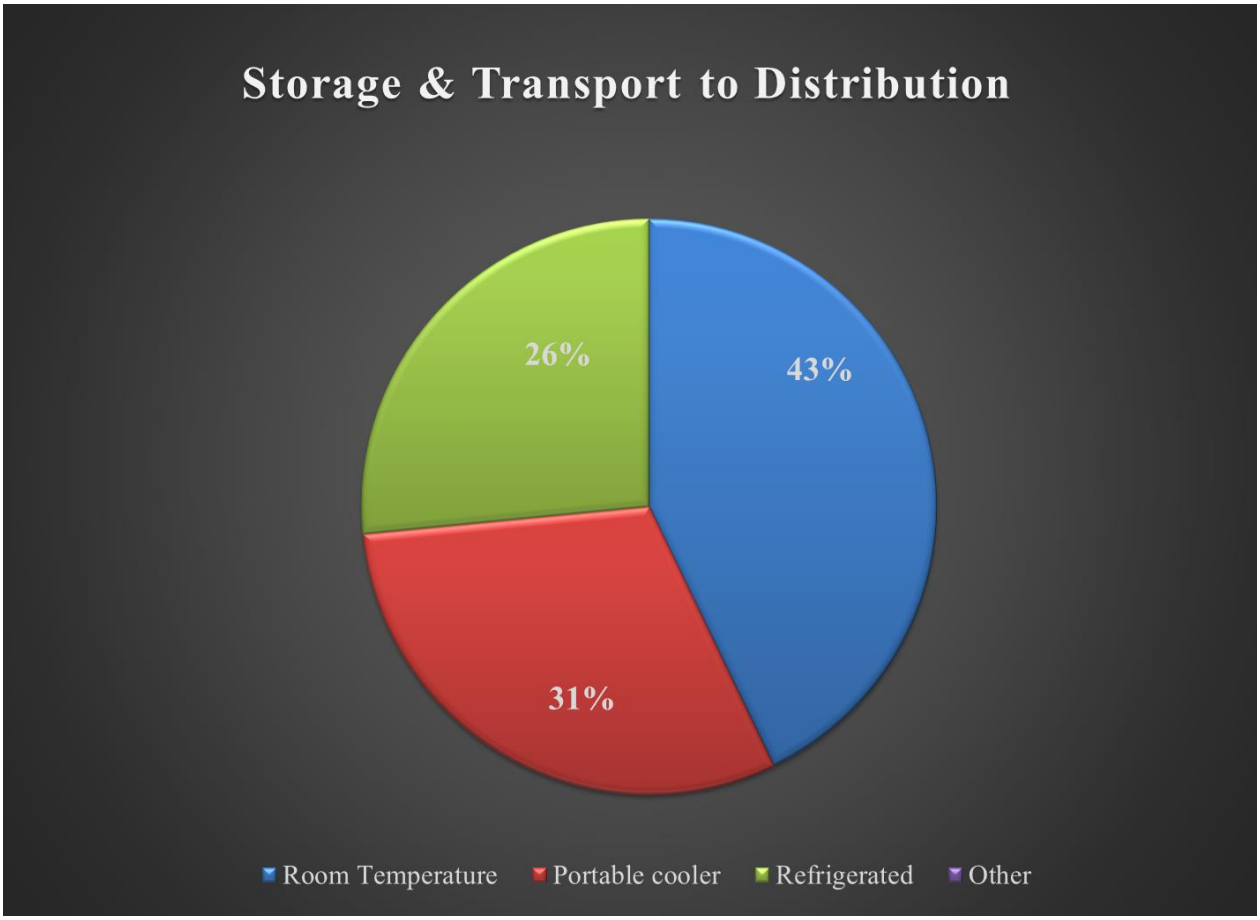

Figure 6.17: Storage and transportation to distribution conditions of non-commercial shell egg suppliers (n=46).

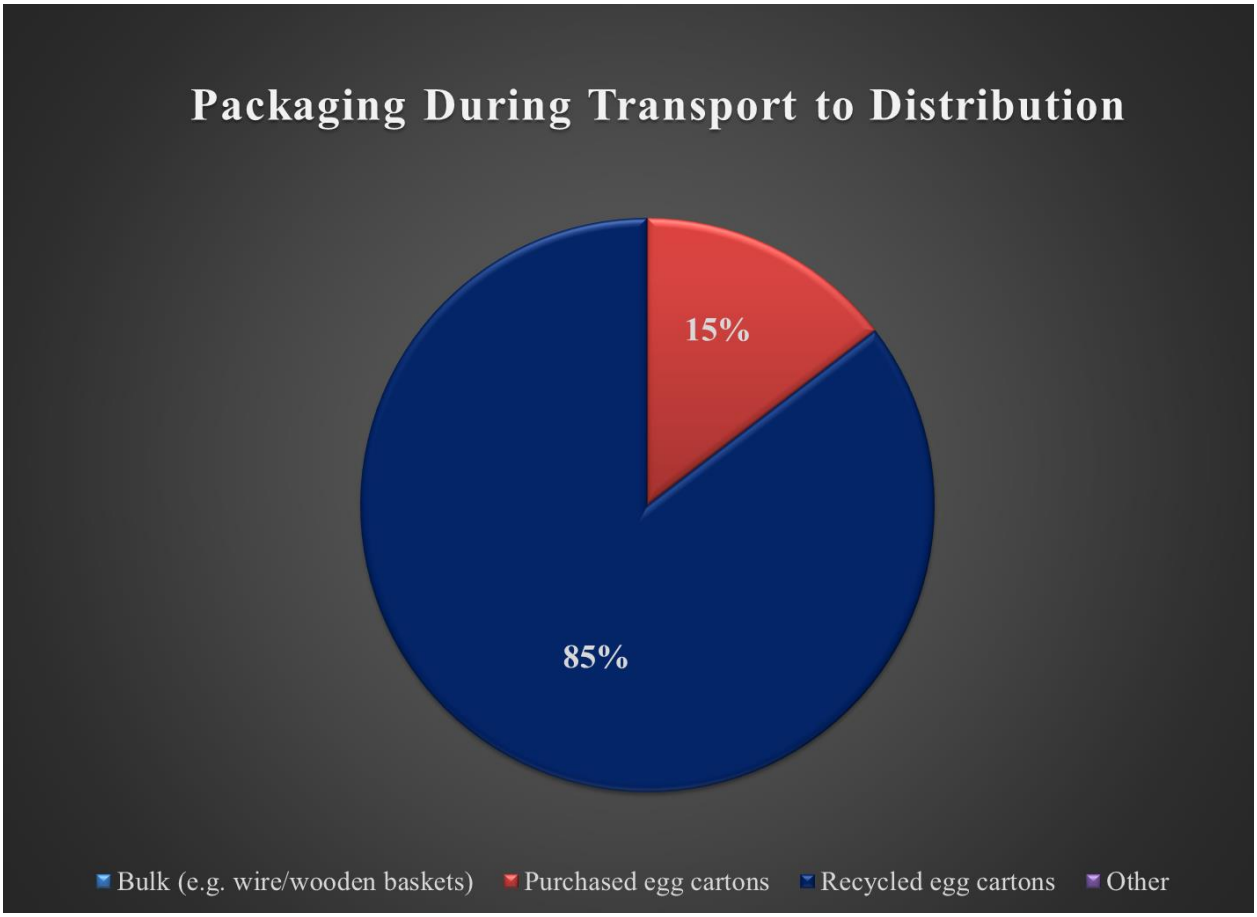

Figure 6.18: Packaging during transport to distribution of non-commercial shell egg suppliers (n=46).

6.6 Conclusions

Non-commercial shell egg suppliers are varied in their flock management and egg handling practices. The variances can contribute to the quality and safety of shell eggs, and is important to consider as a consumer to understand the potential impact to the shell eggs being purchased/obtained. Also, the distribution reach of non-commercial shell egg suppliers can be of important impact to consumer choices. Furthermore, the data realized in this study suggests the need for the additional distribution and training in egg handling.

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APPENDIX

Small and Backyard Layer Flock Survey

Thank you for your participation in this survey. The goal is to better understand the service needs of small and backyard flock owners surrounding egg production, handling, storage, and distribution. The survey will approximately take 15 minutes. Please note the information you provide will be kept confidential and your anonymity is assured. The results you provide will assist in development of information materials to ensure the highest quality and safety of eggs from small and backyard flock owners. Upon completion, please forward the survey to the address below.

Jean Weese, PhD, Professor, Food Safety Extension Specialist,
and Associate Director of Food Systems Institute
Geraldine L. Santos-Norris, Graduate Student
AUBURN UNIVERSITY
Auburn, AL 36849

1) How would you classify your community environment?

☐Urban ☐Suburban ☐Rural

2) What is the size of your layer flock (number of laying chickens owned at time of survey)?

☐1-10 ☐11-20 ☐21-40 ☐41-60 ☐61-80 ☐81-100 ☐>100

3) What is the source of your current flock? (check all that apply)

☐Family/Friends/Neighbors ☐Rescue group
☐Feed Store ☐Internet Site
☐Other, please specify locations below:

_____

4) Do you keep any other animals besides chickens (e.g. farms animals, pets)?

☐No
☐Yes. If so, please specify types and quantity below:

_____

5) Besides eggs, do you produce any other agriculture products? (check all that apply)

- ☐ Row crops (e.g. peanuts, soybean, corn, cotton)
- ☐ Produce
- ☐ Dairy products
- ☐ Broilers
- ☐ Other, please specify products below:

---

6) What type of housing do you use for your flock?

- ☐ Shed/coop with run
- ☐ Shed/coop with free-range restrictions (e.g. during daytime)
- ☐ Cages
- ☐ No housing, unrestricted free-range
- ☐ Other, please specify/describe below:

---

7) What kind of feed does your flock usually receive?

- ☐ Ration purchased at feed store
- ☐ Mixed ration (e.g. purchased feed/kitchen scraps)
- ☐ Kitchen scraps, only
- ☐ Homemade grain-based formulation
- ☐ NONE, chickens gain all nutrients from the range

8) How often do provide calcium supplements?

- ☐ Daily
- ☐ Occasionally
- ☐ Weekly
- ☐ Never

9) What medications have you administered to your layer flock over the past 12 months (check all that apply):

- ☐Coccidiosis preventative
- ☐Deworming
- ☐Antibiotics
- ☐None
- ☐Other, please specify:

_____

10) Have you utilized veterinary services for your flock over the past 12 months?

- ☐No
- ☐If YES, please specify the reason and service provided:

_____

11) Does your layer flock have access to nest boxes?

- ☐No
- ☐If YES, please specify how many: _____

12) How often do you collect eggs?

- ☐Once, daily
- ☐Twice, daily
- ☐Every other day
- ☐Other, please specify how often:

_____

13) Are eggs washed after collection?

- ☐No
- ☐Yes, please specify how:

_____

14) Do you candle the eggs?

☐No

☐If Yes, please specify why:

_____

15) Beyond household consumption, how are surplus eggs distributed? (check all that apply)

☐Neighbors, friends, community

☐Restaurants

☐Schools

☐Farmer's markets

☐Specialty/grocery

☐ Other, please specify below:

_____

16) Approximately how many surplus eggs do you sell a week? _____

17) How are eggs stored for transportation and distribution?

☐Room Temperature

☐Refrigerated

☐Portable cooler

☐Other, please specify technique below:

_____

18) How are eggs packaged for transportation and distribution? (check all that apply)

☐Bulk (e.g. wire/wooden baskets)

☐Purchased egg cartons

☐Recycled egg cartons

☐Other, please specify technique below:

The following inquires regard demographic information:

19) What is your age?

☐19-24 ☐25-34 ☐ 35-44 ☐ 45-54 ☐55-64 ☐65-74
☐75+

20) What is your ethnic origin?

☐African American ☐Asian ☐Caucasian
☐Hispanic/Latino ☐Native American ☐Other, please specify: _____

21) What is the highest level of education you have completed?

☐Some High School ☐High School/GED
☐Trade/Vocational Training
☐Some College ☐2 year College Degree ☐4 year College Degree
☐Graduate Degree