Comparison of fatty acid, cholesterol, and vitamin A and E composition in eggs from hens housed in conventional cage and range production facilities

K. E. Anderson[1]

Department of Poultry Science, North Carolina State University, Raleigh 27695-7608

ABSTRACT The public perceives that the nutritional quality of eggs produced as free range is superior to that of eggs produced in cages. Therefore, this study compared the nutrient content of free-range vs. cage-produced shell eggs by examining the effects of the laboratory, production environment, and hen age. A flock of 500 Hy-Line Brown layers were hatched simultaneously and received the same care (i.e., vaccination, lighting, and feeding regimen), with the only difference being access to the range. The nutrient content of the eggs was analyzed for cholesterol, n-3 fatty acids, saturated fat, monounsaturated fat, polyunsaturated fat, β-carotene, vitamin A, and vitamin E. The same egg pool was divided and sent to 4 different laboratories for analysis. The laboratory was found to have a significant effect on the content of all nutrients in the analysis except for cholesterol. Total fat content in the samples varied ($P < 0.001$) from a high of 8.88% to a low of 6.76% in laboratories D and C, respectively. Eggs from the range production environment had more total fat ($P < 0.05$), monounsaturated fat ($P < 0.05$), and polyunsaturated fat ($P < 0.001$) than eggs produced by caged hens. Levels of n-3 fatty acids were also higher ($P < 0.05$), at 0.17% in range eggs vs. 0.14% in cage eggs. The range environment had no effect on cholesterol (163.42 and 165.38 mg/50 g in eggs from caged and range hens, respectively). Vitamin A and E levels were not affected by the husbandry to which the hens were exposed but were lowest at 62 wk of age. The age of the hens did not influence the fat levels in the egg, but cholesterol levels were highest ($P < 0.001$) at 62 wk of age (172.54 mg/50 g). Although range production did not influence the cholesterol level in the egg, there was an increase in fat levels in eggs produced on the range.

Key words: cage, chicken, egg nutrient composition, range

2011 Poultry Science 90:1600–1608
doi:10.3382/ps.2010-01289

INTRODUCTION

Today's consumers have an increased desire for food produced in more extensive production systems, such as range egg production, because of concerns about the use of the cage environment. The commercial egg industry is responding to these concerns by increasing the production of eggs in cage-free and range settings (Patterson et al., 2001). Anderson (2009a) indicated that currently, the knowledge base on how alternative production methods influence several egg performance, quality, and nutrient characteristics is limited or is based on research conducted many years ago (Lee, 1949; Jull, 1951). Such studies have not been conducted on today's layers or in controlled settings relevant to US egg producers. Comparisons from the European community have indicated no significant nutritional advantage of eggs produced by range chickens over those produced by chickens maintained in cages (Hidalgo et al., 2008).

The nutrient composition of eggs is important to consumers and to commercial egg producers. Publications in the popular press have espoused the view that eggs coming from the range setting have an improved nutritional value (Long and Alterman, 2007; Long and Newbury, 2008). The premise of these survey articles is based on an analysis of range eggs from 14 pastured flocks that were rotated frequently to ensure that the hens had access to fresh pasture. The analysis of the eggs from the survey were then compared with the USDA Nutrient Database for shell eggs. The difficulty with these survey results is that the type of hen and the dietary supplements the range hens received were not known, nor was a concurrent sample of cage-produced eggs collected. In the solicitation of samples to be used in the Mother Earth News (2007) survey, a specific laboratory where the samples were to be sent was specified. Therefore, the goal of the current study was to examine the effect of range or cage production

Received December 9, 2010.
Accepted April 9, 2011.
[1]Corresponding author: ken_anderson@ncsu.edu

Table 1. Seasonal nutrient composition[1] of the bermudagrass, fescue, and clover forage mix in the paddock

Item	Warm season	Cool season
Height (mm)	147.6 ± 18.4	229.4 ± 18.4**
Resting height (mm)	10.4 ± 8.1	43.7 ± 7.0**
Paddock DM (%)	32.6 ± 3.8	32.0 ± 3.3
Sample DM (%)	98.4 ± 0.4	98.1 ± 0.4
CP (%)	15.4 ± 1.6	17.9 ± 1.6
Unavailable protein (%)	1.5 ± 0.2	2.1 ± 0.2
Acid detergent fiber (%)	34.5 ± 3.2	30.4 ± 3.2
Calcium (%)	0.5 ± 0.1	0.6 ± 0.1
Phosphorus (%)	0.3 ± 0.03	0.4 ± 0.03
Potassium (%)	1.9 ± 0.3	2.5 ± 0.3

[1]Nutrient analysis conducted at the North Carolina Department of Agriculture and Consumer Services, Food and Drug Protection Division, Forage Laboratory, Raleigh.

**$P < 0.01$.

Table 2. Soil sample analysis[1] of the paddock area before the beginning of the study

Item	Amount
Humic matter (%)	0.56
Weight/volume (g/cm^3)	1.02
Cation exchange capacity (CEC)	8.9
CEC by basic cations (%)	84.0
CEC occupied by calcium (mg/kg)	996.8
Exchangeable acidity	1.4
pH	6.4
Phosphorus index (mg/kg)	57.6
Potassium index (mg/kg)	86.02
Zinc index (mg/kg)	7.68
Manganese index (mg/kg)	155.84

[1]North Carolina Department of Agriculture and Consumer Services, Agronomic Services, Soil Testing Laboratory, Raleigh.

on egg nutrient content from flocks of hens from the same strain that were hatched simultaneously and that received the same supplemental feed, with the only difference being access to the range. Comparing standard industry husbandry practices with those required in range production settings would offer insight into the effects of each system on the egg nutrient content of cholesterol, total fat, monounsaturated fat, polyunsaturated fat, n-3 fatty acids, β-carotene, vitamin A, and vitamin E. Second, comparing the variability between laboratories would provide information on the laboratories that can be used for nutritional labeling analyses of samples for US Food and Drug Administration and USDA nutritional label requirements.

MATERIALS AND METHODS

The study was conducted concurrently with the North Carolina Layer Performance and Management Test (**NCLP&MT**), which evaluates the major commercial layer lines used in the United States (Anderson, 2009b). Four hundred forty-one Hy-Line Brown pullets were reared in accordance with the laying environment (range or cage) in the 37th NCLP&MT (Anderson, 2007). The rearing dietary program was the same for both the range and cage pullets, with the only difference being access to the range paddock. A general description of the range paddock would be a typical hay mixture for North Carolina consisting of both warm- and cool-season forages. These paddocks were an established bermudagrass and fescue mix and clover. Measurements were taken in accordance with the method of Sharrow (1984) for forage height, and the forage samples were a composite from 5 locations within each paddock. Forage samples were collected twice in each season; the average nutrient composition is shown in Table 1. The only difference between the seasonal samplings was the height ($P < 0.01$) of the forages during the cool season vs. the warm season (229.4 and 147.6 mm, respectively). The resting height logically had a similar distribution. Nutritionally, they were similar in both seasons, indicating that the hens had good year-round forage availability. In addition, a soil sample from the paddock area was collected before the onset of the study and is shown in Table 2. Pullets destined for the range facilities were brooded on litter until 12 wk of age and then moved to the range at 12 wk of age, whereas the cage hens were reared in a cage facility before being moved to the cage laying facility. All other rearing parameters were maintained as similar as possible. When the respective pullets were 17 wk old, the pullet populations were set in each of the 3 range paddocks, with 75 birds in each, and in the cage houses, the pullet populations were assigned to 3 groups of cages, each containing 72 birds. During the laying phase, from 17 through 82 wk of age, the hens were maintained in accordance with the NCLP&MT housing and phase-feeding program (Anderson, 2009b). The range hens were in a range hut that provided 929 cm^2/pullet, 13 cm of roosting space/pullet, and 1 nest/8 hens. The range hut had a timer and light powered via a solar cell with a storage battery to maintain a 16L:8D lighting cycle, which was the same lighting program used in the cage facility. A supplemental propane heater was provided in the range hut for winter conditions, which was maintained at an interior temperature above 7.2°C (45°F), the lowest temperature in the chicken's effec-

Table 3. Name and address of laboratories used for the egg nutrient analysis (in alphabetical order)

Laboratory	Address
Bodycote Testing Group[1]	12003 NE Ainsworth Circle, Suite 105, Portland, OR 97220
Covance	3301 Kinsman Boulevard, Madison, WI 53704
Medallion Laboratories	9000 Plymouth Avenue North, Minneapolis, MN 55427
Silliker Inc.	1304 Halsted Street, Chicago Heights, IL 60411

[1]Currently known as Exova.

Table 4. Analytical procedures used by the laboratories for egg nutrient composition

	Method								
Laboratory[1]	Total fat (g/100 g)	Saturated fat (g/100 g)	Monounsaturated fat (g/100 g)	Polyunsaturated fat (g/100 g)	n-3 (g/100 g)	Cholesterol (mg/100 g)	Vitamin A (IU/100 g)	β-Carotene (mg/100 g)	Vitamin E (IU/100 g)
Bodycote Testing Group[2]	AOAC (2005e), method 969.33 (modified)	AOAC (2005e), method 969.33 (modified)	AOAC (2005e), method 969.33 (modified)	AOAC (2005e), method 969.33 (modified)	Gas chromatography	AOAC (2005i), method 976.26 (modified)	AOAC (2005j), method 981.17 (modified)	AOAC (2005j), method 981.17 (modified)	AOAC (2005g), method 971.30
Covance	AOAC (2005l), method 996.06 (modified); AOCS (2005), method Ce 1h-05	AOAC (2005l), method 996.06 (modified); AOCS (2005), method Ce 1h-05	AOAC (2005l), method 996.06 (modified); AOCS (2005), method Ce 1h-05	AOAC (2005l), method 996.06 (modified); AOCS (2005), method Ce 1h-05	Calculated	AOAC (2000b), method 994.10	AOAC (2005h), method 974.29	AOAC (2000a), method 941.15	McMurray et al. (1980); Cort et al. (1983); Speek et al. (1985);
Medallion Laboratory	AOAC (2005l), method 996.06 [see also AOAC (2005a,c,d,k), methods 920.39C, 933.05, 948.15, 983.23, fat in various foodstuffs]	AOAC (2005l), method 996.06 [see also AOAC (2005a,c,d,k), methods 920.39C, 933.05, 948.15, 983.23, fat in various foodstuffs]	AOAC (2005l), method 996.06 [see also AOAC (2005a,c,d,k), methods 920.39C, 933.05, 948.15, 983.23, fat in various foodstuffs]	AOAC (2005l), method 996.06 [see also AOAC (2005a,c,d,k), methods 920.39C, 933.05, 948.15, 983.23, fat in various foodstuffs]	Calculated separately	AOAC (2005f), method 970.51 (modified)	AOAC (2005m), method 2005.07	AOAC (2005m), method 2005.07	AACC International (2000), method 86-06
Silliker Inc.	AOAC (2005b), method 925.32	AOAC (2005l), method 996.06	AOAC (2005l), method 996.06	AOAC (2005l), method 996.06	Calculated	AOAC (2000b), method 994.10	Reynolds and Judd (1984)	Reynolds and Judd (1984)	AOAC (1995), method 992.03

[1]Each of the laboratories provides analytical reports to food companies for US Food and Drug Administration and USDA nutritional labeling.

[2]Currently known as Exova.

Table 5. Effect of testing laboratory, housing type, and hen age on the nutrient composition analysis of 50-g whole-egg samples

Item	Total fatty acids (%)	Saturated fatty acids (%)	Monounsaturated fatty acids (%)	Polyunsaturated fatty acids (%)	n-3 fatty acids (mg/50 g)	Cholesterol (mg/50 g)	Vitamin A (IU/50 g)	β-Carotene (IU/50 g)	Vitamin E (IU/50 g)
Source									
Laboratory									
A	8.45	2.84^{a}	4.16^{A}	1.46	175.55	159.25	173.97	10.82^{A}	1.03
B	7.88	2.50^{c}	3.69^{B}	1.27	51.00	163.36	145.31	3.34^{C}	1.81
C	6.76	2.17^{d}	3.10^{C}	1.08	27.00	165.86	144.39	6.35^{B}	1.25
D	8.88	2.69^{b}	4.00^{A}	1.42	56.00	169.61	169.47	6.11^{B}	1.14
Pooled SEM	0.12	0.047	0.058	0.30	5.45	2.74	6.47	0.87	0.03
Housing type									
Cage	7.88^{b}	2.55	3.67^{b}	1.25^{B}	70.56^{b}	163.42	160.42	2.77^{B}	1.30
Range	8.11^{a}	2.55	3.80^{a}	1.36^{A}	84.31^{a}	165.38	156.15	10.54^{A}	1.31
Pooled SEM	0.082	0.033	0.041	0.021	3.85	1.94	4.57	0.61	0.02
Hen age									
50 wk	7.94	2.58	3.76	1.27	71.00	160.48^{B}	185.75	6.21^{B}	1.37
62 wk	8.10	2.52	3.72	1.44	99.00	172.54^{A}	121.02	3.51^{C}	1.19
74 wk	7.95	2.56	3.73	1.22	62.50	160.54^{B}	168.08	10.24^{A}	1.36
Pooled SEM	0.20	0.04	0.05	0.03	4.70	2.38	5.60	0.75	0.03
P-value									
Laboratory × hen age	*	NS	NS	****	****	****	**	NS	****

$^{a-d}$Superscripts within a source and column that are different represent a significant difference ($P < 0.05$).
$^{A-C}$Superscripts within a source and column that are different represent a significant difference ($P < 0.0001$).
*$P < 0.05$; **$P < 0.01$; ****$P < 0.0001$.

tive thermal neutral zone at which the body temperature can be maintained via an increase in feed intake. The hens had access to the outdoors throughout the day and appeared to return to the range hut during the dark for roosting and protection (based on observations of the bird care technician). The range pens were 21.3 × 21.3 m (70 × 70 ft) and were enclosed by a fence 1.8 m (6 ft), with the lower chain link section being 1.2 m (4 ft). The perimeter of each paddock was surrounded by a fence and an electric wire that was located 15 cm out from the fence and 15 cm off the ground, which was very effective at controlling access to ground predators. A grid of colored twine was stretched across the paddocks in a random pattern to inhibit aerial predation. Range density was based on a 500 hen/acre static equivalency of 8.04 m^2/hen. The 3 cage replicate groups were housed in cages of standard height in a totally enclosed forced-ventilation laying house with quad-deck cages. Each replicate consisted of 9 cages that were 81.2 cm wide and 40.6 cm deep, which allowed for a density of 413 cm^2, at 8 hens/cage, and the cages were equipped with an automatic feeding system to supply and monitor feed consumption for each individual replicate. The hens were assigned to the replicates in a restricted randomized manner, with the restriction being that all strains should be approximately equally represented in all rows and levels. Husbandry, lighting, and supplemental feed were allocated on the same basis to flock mates in cages and on the range to minimize the variables between flock mates as much as possible. Supplemental feeds were identical between the cage and range hens, and diets were fed ad libitum in accordance with the phase dietary program used in the NCLP&MT (Anderson, 2009b) to provide for optimal performance.

Egg samples were collected at 50, 62, and 74 wk of age during the productive life of the flock, with average egg weights for the range and cage samples of 63.5 and 63.9 g, respectively. The egg samples from the previous 24 h of production were collected from each of the 3 replicate range pens and 3 replicate sets of cages. Six eggs from each replicate were broken into a stomacher bag and then stomached for 60 s. The whole-egg samples were divided into 50-mL conical tubes, as prescribed by the laboratories, and frozen at −29°C. Each of the 18 samples was divided into 4 identical sets, and then shipped on dry ice to the 4 different laboratories used by the commercial egg industry, range egg producers, and the government for nutrient sample analysis in accordance with the sample size needs of each laboratory and the volume needed for each of the tests conducted, with a minimum of two 50-mL samples sent to each laboratory. Each laboratory conducted nutrient analyses for cholesterol, fat, fatty acid profile (including n-3), vitamin A (carotene and retinol), β-carotene, and vitamin E. The laboratories used for the nutrient analysis are shown in Table 3, in alphabetical order. The laboratories selected are used by food manufacturers for the nutrient analysis testing required for labeling,

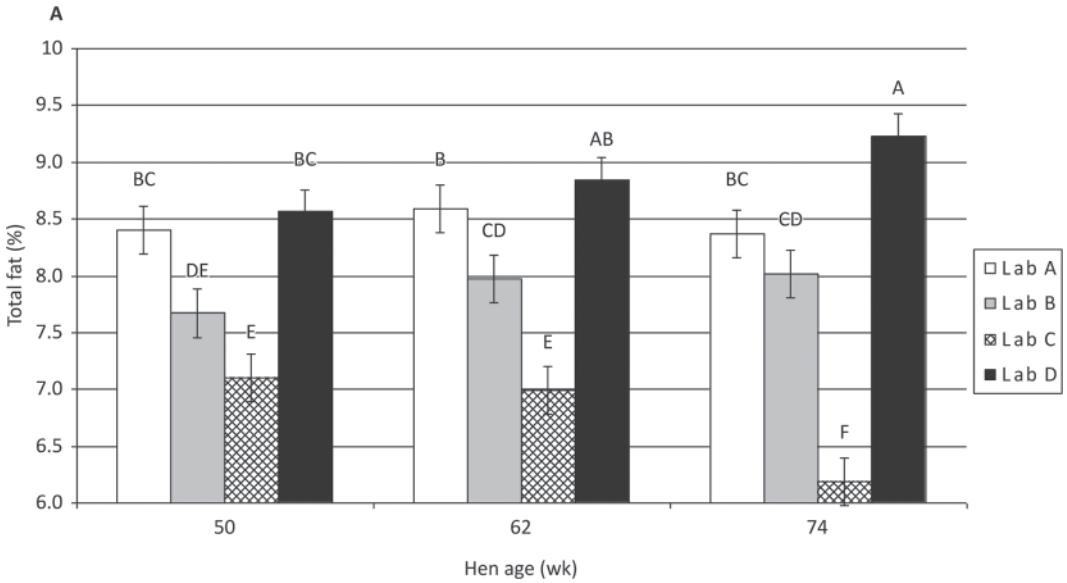

Figure 1. Interactions associated with laboratory × hen age. (A) Total fatty acids; (B) polyunsaturated fatty acids; (C) n-3 fatty acids; (D) cholesterol; (E) vitamin A; (F) vitamin E. Bars with different letters (A–G) are significantly different ($P < 0.05$).

and the analytical methods used by each laboratory are shown in Table 4. All the nutrient analytical procedures and control measures for analysis of quality and reliability are available in greater detail on the web site of each laboratory. One of the laboratories was used for the *Mother Earth News* analysis of range-produced eggs (Long and Alterman, 2007). Laboratory results are not identified with the laboratory in this paper. Results from each laboratory were randomly assigned a label from A to D in random order, as shown in Table 5 and Figures 1A to 1F and in the subsequent discussion.

Experimental Design and Analysis

This experiment was arranged in a factorial design with 3 hen ages, 4 analytical laboratories, and 2 husbandry systems as the factors. We used a single strain of hens under the 2 production environments. Six replicates were sampled at 3 times, for a total of 18 whole-egg samples (n = 18). Data were analyzed using the GLM procedure of SAS (SAS Institute Inc., Cary, NC). The least squares means that were significantly different were separated using the PDIFF option of SAS.

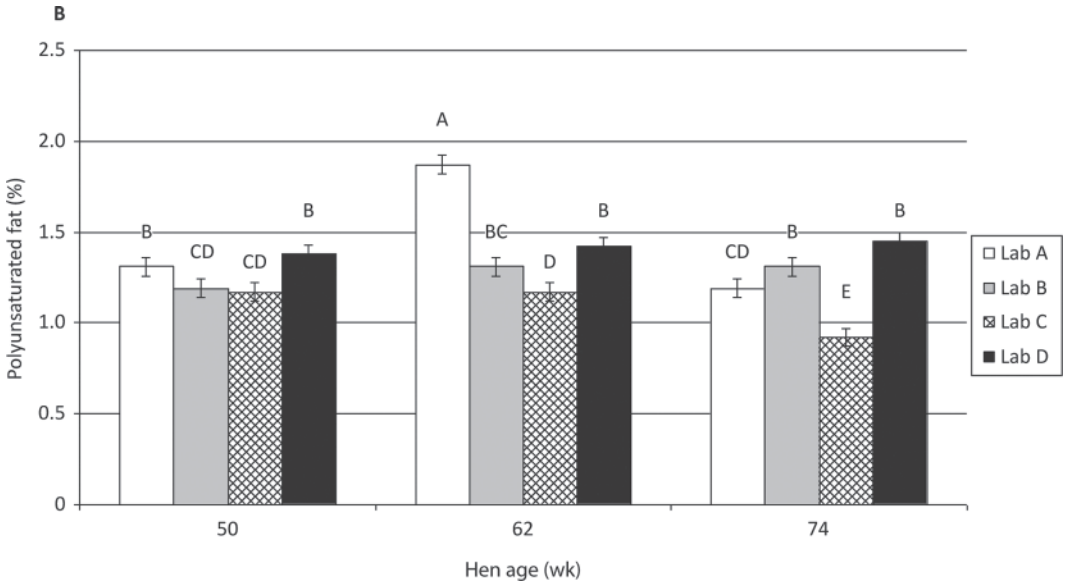

Figure 1 (Continued). Interactions associated with laboratory × hen age. (A) Total fatty acids; (B) polyunsaturated fatty acids; (C) n-3 fatty acids; (D) cholesterol; (E) vitamin A; (F) vitamin E. Bars with different letters (A–G) are significantly different ($P < 0.05$).

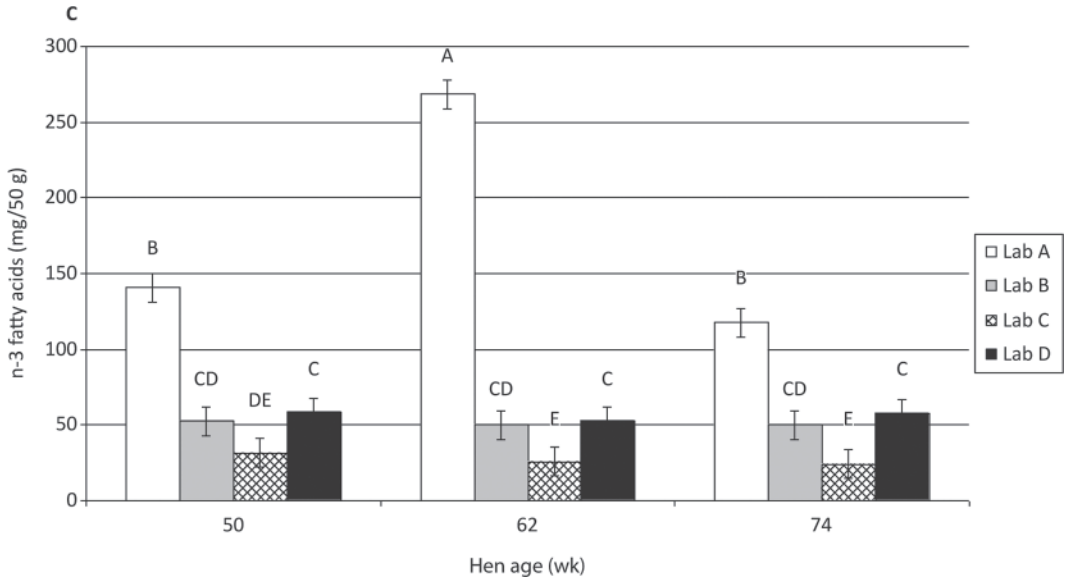

Figure 1 (Continued). Interactions associated with laboratory × hen age. (A) Total fatty acids; (B) polyunsaturated fatty acids; (C) n-3 fatty acids; (D) cholesterol; (E) vitamin A; (F) vitamin E. Bars with different letters (A–G) are significantly different ($P < 0.05$).

RESULTS AND DISCUSSION

Laboratory

The laboratory had a significant effect on the levels of saturated fatty acids, monounsaturated fatty acids, and β-carotene (Table 5). However, there were significant interactions between several variables, as shown in Figure 1. The interaction ($P < 0.05$) of laboratory × hen age on total fat (Figure 1A) showed that laboratories A and D reported a consistently higher total fat content in the samples throughout the sampling periods, which resulted in a high of 8.88% and a low of 6.76%. The interaction occurred when the hens were 74 wk of age, and both laboratories A and C indicated that total fat levels declined, whereas laboratories B and D showed slight increases in total fat. The decline in total fatty acids when the hens were 74 wk old also corresponded to the winter months, when fat intake from insect populations would have been at their lowest. This result may indicate an influence on the fat content of the egg from eating insects (DeFoliart, 1992) and would seem to indicate that it was not the forages or weed seeds but rather the insects that may have caused changes in the fat content of the eggs. However, because not all the laboratories detected this decline

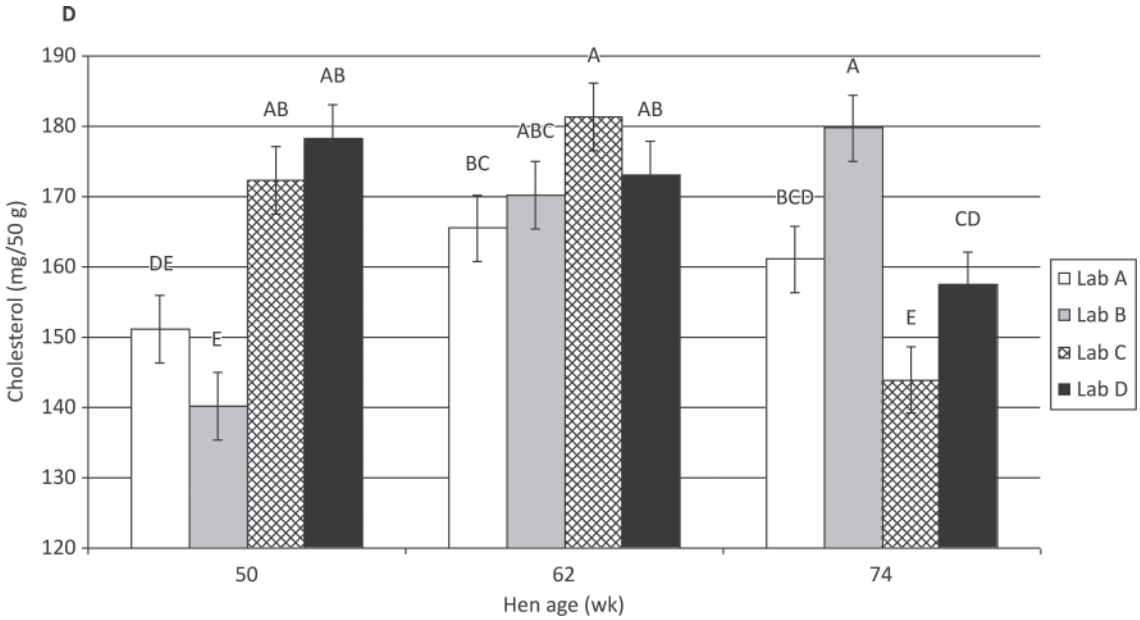

Figure 1 (Continued). Interactions associated with laboratory × hen age. (A) Total fatty acids; (B) polyunsaturated fatty acids; (C) n-3 fatty acids; (D) cholesterol; (E) vitamin A; (F) vitamin E. Bars with different letters (A–G) are significantly different ($P < 0.05$).

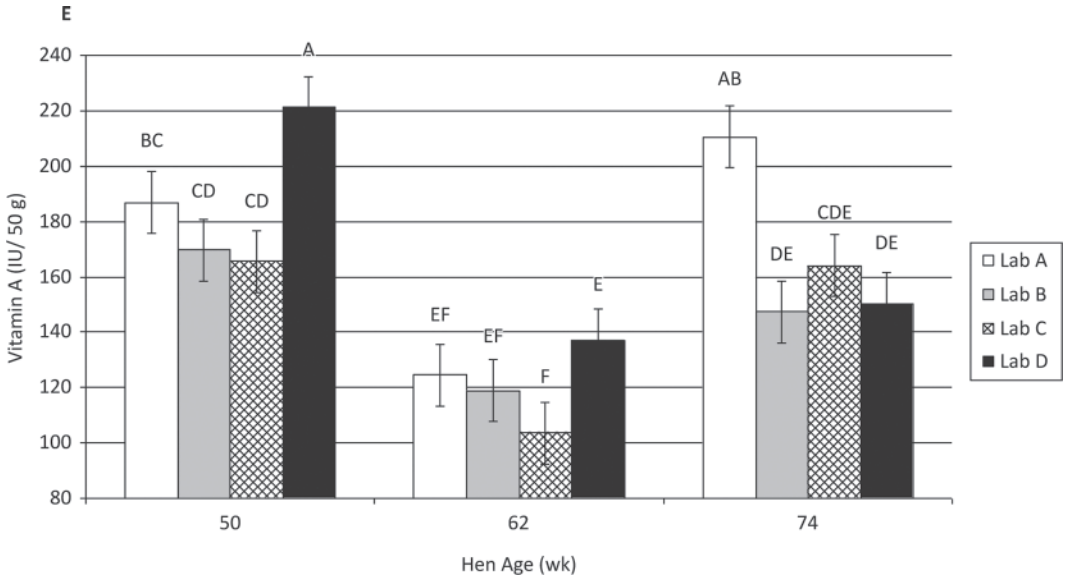

Figure 1 (Continued). Interactions associated with laboratory × hen age. (A) Total fatty acids; (B) polyunsaturated fatty acids; (C) n-3 fatty acids; (D) cholesterol; (E) vitamin A; (F) vitamin E. Bars with different letters (A–G) are significantly different ($P < 0.05$).

among identical samples, the cause for the laboratory differences may be associated with differences in the methodologies used. Because total fatty acids varied among the laboratories, it was somewhat obvious that the percentages of saturated and monounsaturated fats would also vary among laboratories in a similar fashion, with highs of 2.84 and 4.16, and lows of 2.17 and 3.10, respectively. Figure 1B shows the interaction of laboratory × hen age on polyunsaturated fatty acid content ($P < 0.0001$). Laboratory A reported higher polyunsaturates in the egg samples, whereas the other laboratories had similar levels at 62 wk. The level of 175.55 mg/50 g of n-3 fatty acids was highest ($P < 0.01$) in the sample from laboratory A at 62 wk of age, which resulted in a significant interaction ($P < 0.0001$). Analyses of samples from laboratory A were consistently higher in n-3 content than were analyses from the other laboratories (Figure 1C). Laboratory D had the second highest n-3 fatty acid content, at 56.00 mg/50 g. Cholesterol was the most consistent nutrient measured, with a maximum variation of 10.36 mg/50 g of sample, even though a laboratory × hen age interaction ($P < 0.0001$) was observed (Figure 1D). The laboratory analysis at 62 wk showed that the cholesterol analysis from laboratories A and B increased from 50 to 62 wk, whereas the cholesterol levels found by laboratories C

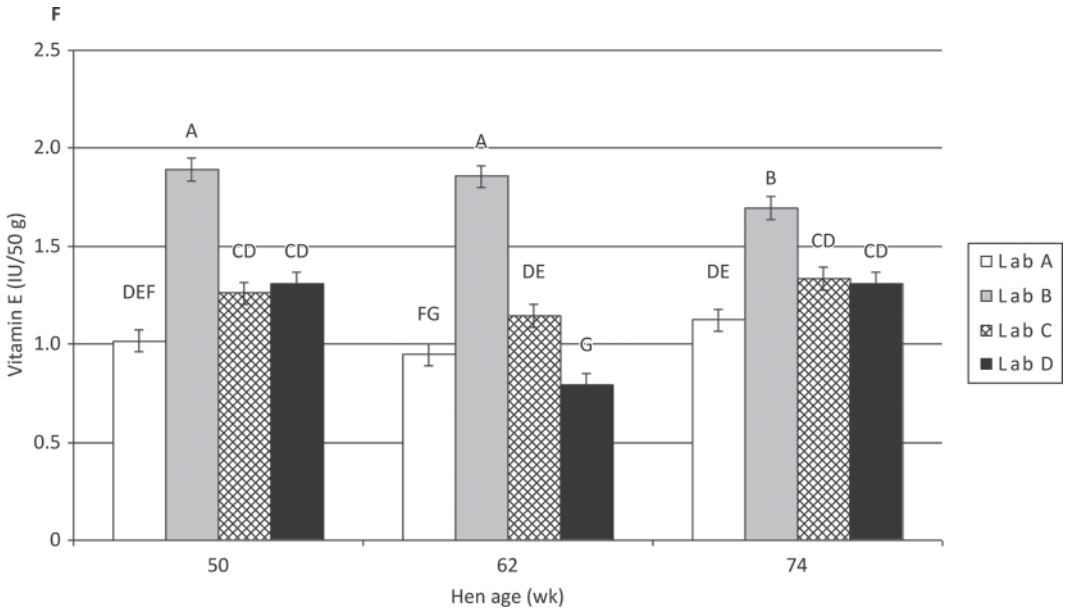

Figure 1 (Continued). Interactions associated with laboratory × hen age. (A) Total fatty acids; (B) polyunsaturated fatty acids; (C) n-3 fatty acids; (D) cholesterol; (E) vitamin A; (F) vitamin E. Bars with different letters (A–G) are significantly different ($P < 0.05$).

and D stayed relatively stable. However, the cholesterol levels reported by laboratories C and D declined ($P < 0.01$) from 62 to 74 wk. Vitamin A was lowest in samples analyzed at laboratories B and C and highest in samples from laboratories A and D. This is shown in the interaction in Figure 1E, where laboratory A at 74 wk and laboratory D at 50 wk had the highest ($P < 0.01$) levels. This was not the case with β-carotene or vitamin E. Vitamin E levels from laboratory B actually had the highest reported values throughout the study period (Figure 1F). The differences in laboratory analyses were not unexpected because the geographic location, equipment used, and procedures used in the laboratories were different, so it was logical that differences would exist. It was also determined that the overall analyzed levels of nutrients in the samples were different among the laboratories that conducted the nutrient analysis (Table 5).

Housing Type

The eggs from the range production system had higher total fat ($P < 0.05$), monounsaturated fat ($P < 0.05$), and polyunsaturated fat ($P < 0.001$) than the eggs produced by caged hens. The higher fat content in the eggs from the range production environment than in the eggs produced by caged hens is an interesting finding. This raised the question of what component in the range resulted in the increased total fat. The logical contributors were forage consumption, an increased intake of wild seeds or insects associate with North Carolina pastures, or both (Bambara and Watson, 2011). DeFoliart (1992) and Banjo, et al. (2006) indicated that there are species of edible insects with fat levels as high as 31.4%. The n-3 levels were also higher ($P < 0.05$), at 84.5 mg/50 g in the range eggs compared with 70.50 mg/50 g in the cage eggs. Even though n-3 levels were higher by a value of 14 mg/50 g, the question was whether this constituted a viable nutrient increase. Having laying hens on the range had no effect on cholesterol, with 160.42 mg/50 g in eggs from cage hens and 156.15 mg/50 g in eggs from range hens. Based on the report by Long and Newbury (2008) on the nutritional advantage of range-produced eggs, it was surprising that virtually no difference in cholesterol content was found between the range- and cage-produced eggs. Both the cage and range housing types had eggs with a lower cholesterol content (by approximately 50 mg/50 g of egg sample) than the nutrient guidelines published by the USDA (USDA-Agricultural Research Service, 2005). Vitamin A and vitamin E levels in eggs were not influenced by housing type in this study. However, β-carotene levels were higher ($P < 0.001$) in the range eggs. Yolk color was not measured, but higher β-carotene levels in the range eggs may have contributed to the observed darker yolks. Even though the range environment did not contribute to the vitamin content, the increased β-carotene may have contributed to increases in lycopene, lutein, or zeaxanthin, none of which were measured in this study.

Hen Age

The age of the hen did not affect the total fat percentage or other fat components. Cholesterol levels were 12 mg higher ($P < 0.001$) at 62 wk than at 50 or 74 wk. It is interesting to note that the vitamin A content declined ($P < 0.05$) as the hens aged, from a high of 185.75 IU/50 g at 50 wk to a low of 121.02 IU/50 g at 62 wk, with intermediate levels at 74 wk (Figure 1E). In contrast, the β-carotene content of the egg was at its lowest at 62 wk and reached its highest at 74 wk. The levels at 62 wk corresponded to the fall season, when the amount of lush forage would be limited after the hot, dry summer season, followed by the resurgence of forages in the cooler winter months when the forages had the opportunity to recover, thereby providing more carotenes in the diet of the hens on the range.

In this study, a significant nutritional advantage of eggs produced by range chickens over eggs produced by chickens maintained in cages could not be established, which is similar to the results of Hidalgo et al. (2008). The range eggs had higher fat levels, including for n-3 (13.8 mg/50 g), but this would not be viewed as a nutritional advantage, and, in fact, may negatively affect some functional components of the egg when used in recipes. Eggs from birds in the range system may have elevated levels of lycopene, lutein, and zeaxanthin, as indicated by the elevated β-carotenes, but this was not verified in a laboratory analysis. This study indicates that the USDA-Agricultural Research Service (2005) nutritional guidelines should be evaluated for eggs; however, this study did not examine the effect of molting or the strain of laying hen on the nutrient content of the eggs.

REFERENCES

AACC International. 2000. Method 86-06: Analysis of vitamins A and E by high-performance liquid chromatography. In AACC International Approved Methods of Analysis. AACC Int., St. Paul, MN.

Anderson, K. E. 2007. Report on Pullet Rearing Period: 37th North Carolina Layer Performance and Management Test. Vol. 37, No. 2, December 2007. North Carolina State University Cooperative Extension Service, Raleigh. Accessed Mar. 30, 2009. http://www.ces.ncsu.edu/depts/poulsci/tech_manuals/layer_reports/37_grow_report.pdf.

Anderson, K. E. 2009a. Overview of natural and organic production: Looking back to the future. J. Appl. Poult. Res. 18:348–354.

Anderson, K. E. 2009b. Single Production Cycle Report of the Thirty Seventh North Carolina Layer Performance and Management Test. Vol. 37, No. 4, February 2009. North Carolina State University Cooperative Extension Service, Raleigh. Accessed Mar. 30, 2009. http://www.ces.ncsu.edu/depts/poulsci/tech_manuals/layer_reports/37_single_cycle_report.pdf.

AOAC. 1995. AOAC Official Method 992.03: Vitamin E activity (all-*rac*-tocopherol) in milk and milk-based infant formula. Liquid Chromatographic Method. In Official Methods of Analysis of the Association of Official Analytical Chemists. 16th ed. Assoc. Off. Anal. Chem., Arlington, VA.

AOAC. 2000a. AOAC Official Method 941.15: Carotene in fresh plant materials and silages. Spectrophotometric Method. In Official Methods of Analysis of the Association of Official Analytical Chemists. 17th ed. Assoc. Off. Anal. Chem., Arlington, VA.

AOAC. 2000b. AOAC Official Method 994.10: Cholesterol in foods. In Official Methods of Analysis of the Association of Official Analytical Chemists. 17th ed. Assoc. Off. Anal. Chem., Arlington, VA.

AOAC. 2005a. AOAC Official Method 920.39C: Gravimetry (ether extraction) I. Pearl millet flour fibre, crude. ISO 5498:1981. In Official Methods of Analysis of the Association of Official Analytical Chemists. 18th ed. Assoc. Off. Anal. Chem., Arlington, VA.

AOAC. 2005b. AOAC Official Method 925.32: Fat in eggs. In Official Methods of Analysis of the Association of Official Analytical Chemists. 18th ed. Assoc. Off. Anal. Chem., Arlington, VA.

AOAC. 2005c. AOAC Official Method 933.05: Fat in cheese. In Official Methods of Analysis of the Association of Official Analytical Chemists. 18th ed. Assoc. Off. Anal. Chem., Arlington, VA.

AOAC. 2005d. AOAC Official Method 948.15: Fat in seafood. In Official Methods of Analysis of the Association of Official Analytical Chemists. 18th ed. Assoc. Off. Anal. Chem., Arlington, VA.

AOAC. 2005e. AOAC Official Method 969.33. 1: Fatty acids in oils and fats. Preparation of methyl esters. Boron trifluoride method. In Official Methods of Analysis of the Association of Official Analytical Chemists. 18th ed. Assoc. Off. Anal. Chem., Arlington, VA.

AOAC. 2005f. AOAC Official Method 970.51: Fats (animal) in vegetable fats and oils (determination of cholesterol). In Official Methods of Analysis of the Association of Official Analytical Chemists. 18th ed. Assoc. Off. Anal. Chem., Arlington, VA.

AOAC. 2005g. AOAC Official Method 971.30: α-Tocopherol and α-tocopheryl acetate in foods and feeds. Colorimetric method. In Official Methods of Analysis of the Association of Official Analytical Chemists. 18th ed. Assoc. Off. Anal. Chem., Arlington, VA.

AOAC. 2005h. AOAC Official Method 974.29: Vitamin A in mixed feeds, premixes, and human and pet foods. Colorimetric Method. In Official Methods of Analysis of the Association of Official Analytical Chemists. 18th ed. Assoc. Off. Anal. Chem., Arlington, VA.

AOAC. 2005i. AOAC Official Method 976.26: Cholesterol in multicomponent foods. Gas chromatographic method. In Official Methods of Analysis of the Association of Official Analytical Chemists. 18th ed. Assoc. Off. Anal. Chem., Arlington, VA.

AOAC. 2005j. AOAC Official Method 981.17: Vitamin D in fortified milk and milkpowder. Liquid chromatographic method. In Official Methods of Analysis of the Association of Official Analytical Chemists. 18th ed. Assoc. Off. Anal. Chem., Arlington, VA.

AOAC. 2005k. AOAC Official Method 983.23: Fat in foods. Chloroform-methanol extraction method. In Official Methods of Analysis of the Association of Official Analytical Chemists. 18th ed. Assoc. Off. Anal. Chem., Arlington, VA.

AOAC. 2005l. AOAC Official Method 996.06: Fats (total, saturated, and monounsaturated in foods). Hydrolytic Extraction. Gas Chromatographic Method. In Official Methods of Analysis of the Association of Official Analytical Chemists. 18th ed. Assoc. Off. Anal. Chem., Arlington, VA.

AOAC. 2005m. AOAC Official Method 2005.07: β-Carotene in supplements and raw materials. Reversed-phase high pressure liquid chromatographic method. In Official Methods of Analysis of the Association of Official Analytical Chemists. 18th ed. Assoc. Off. Anal. Chem., Arlington, VA.

AOCS. 2005. AOCS Official Method Ce 1h-05: Determination of *cis*-, *trans*-, saturated, monounsaturated and polyunsaturated fatty acids in vegetable or non-ruminant animal oils and fats by capillary GLC. In Official Methods and Recommended Practices of the AOCS. Am. Oil Chem. Soc., Urbana, IL.

Bambara, S., and D. W. Watson. 2011. Insects found in forage and pasture. North Carolina State University Cooperative Extension Service, Department of Entomology, Raleigh. Accessed Mar. 24, 2011. http://www.ces.ncsu.edu/depts/ent/notes/forages/past&for/past&for.html.

Banjo, A. D., O. A. Lawal, and E. A. Songonuga. 2006. The nutritional value of fourteen species of edible insects in southwestern Nigeria. Afr. J. Biotechnol. 5:289–301.

Cort, W. M., T. S. Vicente, E. H. Waysek, and B. D. Williams. 1983. Vitamin E content of feedstuffs determined by high-performance liquid chromatographic fluorescence. J. Agric. Food Chem. 31:1330–1333.

DeFoliart, G. 1992. Insects as human food. Crop Prot. 11:395–399.

Hidalgo, A., M. Rossi, F. Clerici, and S. Ratti. 2008. A market study on the quality characteristics of eggs from different housing systems. Food Chem. 106:1031–1038.

Jull, M. A. 1951. Poultry Husbandry. 3rd ed. McGraw-Hill Book Company, New York, NY.

Lee, C. E. 1949. Profitable Poultry Management. 1949 ed. Beacon Milling Company, Cayuga, NY.

Long, C., and T. Alterman. 2007. Meet real free-range eggs. Mother Earth News. October/November 2007. Accessed Mar. 24, 2009. http://www.motherearthnews.com/Real-Food/2007-10-01/Tests-Reveal-Healthier-Eggs.aspx.

Long, C., and U. Newbury. 2008. The good egg. Mother Earth News. Accessed Feb. 5, 2008. http://www.motherearthnews.com/print-article.aspx?id=74318.

McMurray, C. H., W. J. Blanchflower, and D. A. Rice. 1980. Influences of extraction techniques on determination of α-tocopherol in animal feedstuffs. J. AOAC 63:1258–1261.

Mother Earth News. 2007. How do I get my eggs tested? Accessed Feb. 2008. http://www.motherearthnews.com/eggs/testing.aspx.

Patterson, P. H., K. W. Koelkebeck, D. D. Bell, J. B. Carey, K. E. Anderson, and M. J. Darre. 2001. Egg marketing in national supermarkets: Specialty eggs—Part 2. Poult. Sci. 80:390–395.

Reynolds, S. L., and H. J. Judd. 1984. Rapid procedure for the determination of vitamins A and D in fortified skimmed milk powder using high-performance liquid chromatography. Analyst (Lond.) 109:489–492.

Sharrow, S. H. 1984. A simple disk meter for measurement of pasture height and forage bulk. J. Range Manage. 37:94–95.

Speek, A. J., J. Schrijver, and W. H. P. Schreurs. 1985. Vitamin E composition of some seed oils as determined by high-performance liquid chromatography with fluorometric detection. J. Food Sci. 50:121–124.

USDA-Agricultural Research Service. 2005. USDA National Nutrient Database for Standard Reference, Release 18. Accessed Mar. 30, 2009. http://www.nal.usda.gov/fnic/foodcomp/Data/SR14/reports/sr14fg01.pdf.