

## Article

# Feeding Laying Hens a Diet Containing High-Oleic Peanuts or Oleic Acid Enriches Yolk Color and Beta-Carotene While Reducing the Saturated Fatty Acid Content in Eggs

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**Abstract:** We investigated the dietary effects of high-oleic peanuts (HOPN) or oleic fatty acids (OA) on older production hen performance, egg mass and quality, and lipid composition. A total of 99 laying hens were divided between three treatments and fed ad libitum for 8 weeks: (1) Conventional diet; (2) HOPN diet; (3) OA diet. Body weight (BW) was measured at weeks 1 and 8, and feed, egg weights (EW), and egg quality parameters were collected. Data was analyzed by analysis of variance at  $p < 0.05$  significance level. There were no treatment differences in 8 week BW, feed conversion ratio, or average weekly egg quality parameters. The 8 week average EW of eggs from the HOPN group had reduced EW relative to the other treatment groups ( $p = 0.0004$ ). The 8-week average yolk color score ( $p < 0.0001$ ) was greater in eggs from the HOPN group relative to the other treatments. Overall, the  $\beta$ -carotene ( $p < 0.006$ ) and OA content ( $p < 0.0001$ ) was greater in eggs from the HOPN group, with reduced saturated fats in eggs from the HOPN group relative to the other treatments. These results suggest that HOPN and/or OA may be a useful layer feed ingredient to enrich eggs, while significantly reducing egg size in older production hens.

**Keywords:** alternative feed ingredients; high-oleic peanuts; laying hens; shell eggs

## 1. Introduction

For decades, soybean meal has been utilized globally in poultry diets, with maize providing the primary source of dietary energy [1]. Yet these feed ingredients are often inaccessible to developing countries for poultry production due to high cost and availability of soybean and corn, with the United States, Brazil, and Argentina being the largest producers and exporters of soybeans [1]. Hence, other protein sources like canola meal, peanut meal, fishmeal, and blood meal are also utilized.

In India, Ghana, and Nigeria, peanut meal is commonly utilized as a protein-rich poultry feed ingredient [2–6], yet in the US, 85% of peanut production is for peanut butter and snacks, with the remaining 15% crushed for oil [7]. Early poultry feeding trials established that peanut meal prepared from conventional normal-oleic peanuts (22–30% protein and 44–56% total fat, with a total fatty acid profile of 52% oleic acid and 27% linoleic acid) is a reasonable poultry feed ingredient [8,9]. Additionally, limited studies have investigated the use of high-oleic peanuts (22–30% protein and 44–56% total fat) with an 80% oleic fatty acid and 2% linoleic fatty acid profile as an alternative poultry ration. Toomer et al. [10] reported that eggs produced from Leghorns in peak egg production (40 weeks of lay) fed high-oleic peanuts had increased  $\beta$ -carotene and oleic fatty acid content, with increased yolk color compared to conventional eggs, with no significant differences in hen performance (with the exception of egg mass) or egg quality between

treatment groups. However, egg weights produced from hens in peak egg production (40 weeks of lay) fed a high-oleic peanut diet were significantly smaller in mass relative to conventional eggs at all time points measured [10]. Hence, the primary aim of this project was to determine the effect of feeding a high-oleic peanut or an oleic acid diet on the size/mass of the eggs produced by older production hens (57 weeks of lay). The establishment of a feeding regimen to reduce the production of oversized eggs commonly seen with increasing production age is of great commercial interest [11]. Based on our previous findings [10], we conjecture that eggs produced from hens fed a high-oleic peanut or oleic acid diet will have enhanced  $\beta$ -carotene and unsaturated fatty acid content, with smaller egg size (mass), as compared to eggs produced from hens fed a conventional diet. Moreover, we aimed to investigate the effects of a high-oleic peanut diet on the sensory attributes of the eggs produced.

## 2. Materials and Methods

All animal research procedures used in these feeding trials were approved by the North Carolina State University Institutional Animal Care and Use Committee (IACUC #17-001-A).

### 2.1. Experimental Design, Animal Husbandry, Dietary Treatments, and Hen Performance

All experimental diets were formulated in Concept 4 (level 2, version 10.0) software to be isonitrogenous (18% protein) and isocaloric (3080 kcal/kg metabolizable energy), with an estimated particle size between 800 and 1000  $\mu\text{m}$  (Table 1). The control diet (Treatment 1) was prepared as a conventional layer diet with defatted soybean meal and corn, while Treatment 2 was prepared using aflatoxin-free whole non-roasted unblanched high-oleic peanuts. Peanuts were crushed using a Roller Mill to form crumbles, prior to inclusion in Treatment 2. Treatment 3 was prepared by supplementing the control diet with 2.64% (% by weight) of food-grade oleic fatty acid oil (Millipore Sigma, Burlington, MA, USA). Each of the experimental diets were supplemented with vitamin, mineral, and selenium premixes manufactured at the NC State University Feed Mill (Raleigh, NC, USA) to meet and/or exceed poultry requirements for vitamins, minerals, and selenium. All experimental diets were analyzed by the North Carolina Department of Agriculture and Consumer Services and the Food and Drug Protection Division Laboratory (Raleigh, NC, USA) for aflatoxin and microbiological contaminants.

Brown Leghorn hens were selected for use in this study from the University Flock, NC State University (Raleigh, NC, USA). In total, 99 Brown Leghorn hens (57 week of lay) were assigned to three dietary treatment groups for 8 weeks: (1) Conventional diet; (2) HOPN diet; (3) OA diet. There were three replicates per treatment, with hens individually housed in battery cages (each cage measured 12 inches wide  $\times$  18 inches deep  $\times$  18 inches height) in one room at the Chicken Education Unit, NC State University (Raleigh, NC, USA). Hens were provided feed and water ad libitum and 14 L:D for 8 weeks. Body weights were recorded for each individual hen at week 1 and week 8, with feed weights recorded weekly. Shell eggs were collected, enumerated, and weighed daily. Total number of eggs produced per replicate and per treatment was calculated for each experimental week and for the total 8 week feeding trial. The average feed conversion ratio (FCR) was calculated as total feed consumed over the 8-week feeding (kg)/dozens of eggs produced for each treatment group over the 8-week feeding trial.

### 2.2. Egg Quality and Grading

Bi-weekly (0-week, 2-week, 4-week, 6-week, and 8-week), 36 eggs were randomly selected with 12 shell eggs per treatment (4 eggs randomly selected from each replicate) for quality assessment and USDA grading. Fresh shell eggs were collected on the day of quality assessment and USDA grading. Shell eggs were analyzed for DSM yolk color score, vitelline membrane strength, Haugh unit, and shell strength by the Laying Hen and Small Flock Management Lab, Prestage Department Poultry Science, NC State University.

Haugh unit values were determined using methods described by Haugh [12] and were recorded with the Technical Services and Supplies (TSS) QCD system (Dunnington, York, United Kingdom). The QCD system was calibrated to the DSM Color Fan, consisting of a series of 15 colored plastic tabs with a range of yolk colors from light yellow to orange red (color index 1 to 15), defined by Vuillemier [13]. In general, a texture analyzer (TA.XTplus) was used to measure the shell strength and vitelline membrane strength by the breaking strength using a 5-kg load cell per the manufacturer's instructions (Stable Micro Systems, Surrey, United Kingdom), with measurements in grams of force. Vitelline membrane strength was determined using methods described by Jones et al. (2005), with a 2 mm/second test speed and 0.0001 kg trigger force [14]. Modified methods of Jones et al. (2002) were used to measure shell strength with a 2 mm/second test speed and a 0.001 kg trigger force [15].

**Table 1.** Composition of formulated experimental laying hen diets <sup>1</sup>.

Ingredients	Treatments <sup>2</sup>		
	Control	HOPN	OA
		% (by weight)	
Yellow Corn	46.4	39.0	52.3
Corn Gluten Meal	5.0	10.4	5.0
High-Oleic Peanut <sup>3</sup>	0.0	20.0	0.0
Soybean Oil	7.8	0.0	0.0
Soybean Meal	21.4	0.0	20.4
Wheat Bran	6.0	16.8	6.0
Oleic Acid Oil	0.0	0.0	2.6
Calcium Carbonate	10.9	10.8	11.3
Dicalcium Phosphorus	1.6	1.4	1.5
Sodium Chloride	0.3	0.3	0.3
L-Lysine	0.0	0.5	0.0
DL-Methionine	0.1	0.1	0.1
L-Tryptophan	0.0	0.03	0.0
L-Threonine	0.0	0.13	0.0
Choline Chloride	0.2	0.2	0.2
<sup>4</sup> MYC-Out <sup>TM</sup>	0.1	0.1	0.1
Mineral Premix <sup>5</sup>	0.2	0.2	0.2
Vitamin Premix <sup>6</sup>	0.1	0.1	0.1
Selenium Mix <sup>7</sup>	0.1	0.1	0.1
Metabolizable Energy (kcal/kg)	3080	3080	3080

<sup>1</sup> Three isocaloric, isonitrogenous (18% protein) formulated diets were fed to Brown Leghorn (57 week of lay) hens for 8 weeks. <sup>2</sup> Treatments: control = conventional soybean meal and corn mash diet, HOPN = unblanched high-oleic peanut crumbles (20%) and corn mash diet, OA = control diet supplemented with 2.64% food-grade oleic fatty acid oil. <sup>3</sup> High-Oleic Peanuts = unblanched raw whole high-oleic peanut crumbles. <sup>4</sup> MYC-Out<sup>TM</sup> = mycotoxin binder and feed antioxidant manufactured Adisseo (Alpharetta, GA, USA). <sup>5</sup> Mineral premix, manufactured by NCSU FeedMill, supplied the following per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; and cobalt. <sup>6</sup> Vitamin premix, manufactured by NCSU FeedMill, supplied the following per kg of diet: 13,200 IU vitamin A, 4000 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B12, 0.13 mg biotin, 2 mg menadione (K3), 2 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 4 mg vitamin B6, 55 mg niacin, and 1.1 mg folic acid. <sup>7</sup> Selenium premix, manufactured by NCSU FeedMill, = 1 mg Selenium premix provided 0.2 mg Se (as Na<sub>2</sub>SeO<sub>3</sub>) per kg of diet.

### 2.3. $\beta$ -Carotene, Lipid Content, and Fatty Acid Analysis

All experimental diets were analyzed for lipid content, fatty acid, and  $\beta$ -carotene content in triplicate by an external vendor ATC Scientific (Little Rock, AR, USA), using AOAC approved methods. Gross energy analysis of feed samples was performed by ATC Scientific using an adiabatic oxygen bomb calorimeter with standard methods. Bi-weekly, a total of 45 eggs were randomly selected, with 15 eggs per treatment (5 eggs per replicate) for lipid content,  $\beta$ -carotene, and fatty acid analysis by ATC Scientific using AOAC approved methods. Each egg sample was mixed for homogeneity in a whirl-pak<sup>®</sup> (Millipore Sigma, St. Louis, MO, USA) bag for 3 min using a Smasher<sup>TM</sup> Lab Blender (Weber Scientific, Hamilton, NJ, USA). Subsequently, all egg samples were frozen at  $-20$  °C and

stored frozen until chemical analysis within 2 weeks of collection. Frozen homogenous egg samples were shipped on dry ice overnight to vendor for analysis within 2 weeks of collection. Lipid (total cholesterol, crude fat) and fatty acid analysis of homogenous egg samples and feed samples were analyzed using direct methylation methods, as described by Toomer et al. [10]. Total cholesterol was measured as mg cholesterol/100 g sample weight (feed or egg), while crude fat was measured as a percentage of gram crude fat/gram sample weight (feed or egg). Fatty acid content was measured as a percentage of gram of fatty acid/gram total lipid content of a sample (feed or egg). Methods used to determine  $\beta$ -carotene content in eggs are detailed in the AOAC 958.05 [16] color of egg yolk method. Egg fat hydrolysis methods were determined using the AOAC method 954.02 [17].

#### 2.4. Cooking Methods and Consumer Acceptance Testing of Scrambled Eggs

The Sensory Service Center, in the Food, Bioprocessing, and Nutrition Sciences Department, NC State University (Raleigh, NC, USA), performed all sensory testing and data analysis of egg samples. The sensory protocol was reviewed and deemed exempt by the NC State University Institutional Review Board for human subjects. Scrambled egg samples were prepared following safe food handling practices. During preparation and service, all team members wore gloves, hairnets/hats, and lab coats. Whole shell eggs were received and refrigerated at 4 °C upon arrival. On the day of testing, preparation of raw eggs was completed on a separate table from the cooking/serving areas of scrambled eggs to prevent cross contamination of any microbial hazards, and gloves were changed and hands were frequently washed during any transition from raw to cooked product.

On the day of the sensory evaluation, approximately 150 shell eggs per treatment were cooked. In total, 10 sets of 15 eggs/treatment were cracked into a bowl and beat together until homogenous. A large non-stick pan was heated over medium heat for approximately 1 min. The homogenous egg mixture was added to the heated pan and stirred slowly with a wooden spatula, bringing in the mixture from the edges of the pan for 3.5 min and subsequently removing the eggs from the pan. Scrambled egg samples from each of the three treatments were placed in labeled aluminum pans, covered with aluminum foil, and held in a heated holding cabinet at 180 °F to maintain quality. Scrambled egg portions were dispensed into lidded soufflé cups with three-digit codes to identify treatment for consumer testing.

Self-reported scrambled egg consumers ( $n = 109$ ) were recruited from the NC State University staff and student population. Consumer panelists confirmed no egg food allergies and/or sensitivities prior to participation. Consumer panelists were disqualified if they were younger than 17 years of age, older than 65 years of age, or if they only consumed scrambled eggs once a month. Upon completion of the test, consumer panelists were compensated with a \$5 gift card to a local store. Compusense20 Cloud (Guelph, ON, Canada) was used for data collection and analysis. Samples were presented monadically with a 2-min enforced rest period between egg samples. Consumers evaluated various aroma, flavor, and texture liking attributes using a 9-point hedonic scale, where 1 = dislike extremely and 9 = like extremely. Consumers used a 5-point anchored Just About Right (JAR) scale to evaluate flavor and color attributes. Consumers were provided with spring water and unsalted crackers for palate cleansing.

#### 2.5. Statistical Analysis-Laying Hen Performance and Egg Lipid and Fatty Acid Content

Each hen served as the experimental unit for all performance data. All performance data was evaluated for significance by one-way analysis of variance (ANOVA) at a significance level of  $p < 0.05$  using SAS statistical software (version 9.4). If ANOVA results were significant ( $p < 0.05$ ), Tukey's multiple comparisons t-test was conducted to compare the mean of each treatment group with the mean of every other treatment at  $p < 0.05$  significance level. Comparisons were made between body weights (33 birds/treatment), feed intake (33 birds/treatment), feed conversion ratio (33 birds/treatment), and egg weights (total # of eggs collected over the 8-week feeding trial).

In total, 12 eggs per treatment (4 eggs per replicate randomly selected) were statistically analyzed by one-way ANOVA ( $p < 0.05$ ) using SAS. Means were separated by least squares means, with Tukey-Kramer adjustment for multiple comparisons ( $p < 0.05$ ) for treatment differences in egg quality parameters (Haugh unit, vitelline membrane strength, shell strength, yolk color score) at each bi-weekly experimental time-point (0-week, 2-week, 4-week, 6-week, 8-week). Additionally, eggs were statistically analyzed for treatment differences in egg quality parameters in all eggs collected over the 8-week feeding trial (180 total, 60 eggs per treatment). In total, 15 homogenous egg samples (5 per replicate) were statistically analyzed by one-way ANOVA ( $p < 0.05$ ) using SAS. Means were separated by least squares means with the Tukey-Kramer adjustment for multiple comparisons ( $p < 0.05$ ) for treatment differences in egg  $\beta$ -carotene content and egg lipid and fatty acid content (45 total egg samples at each time-point) weekly (2-, 3-, 4-, 5-, 6-, 7-, and 8-week).

### 2.6. Statistics-Sensory Evaluation

Statistical analysis was conducted using XLSTAT software (version 2016; Addinsoft, New York, NY, USA). Descriptive analysis results and consumer liking scores were analyzed by ANOVA, with Fisher's least significant difference test at a significance level of  $p < 0.05$ . Principal component analysis was applied to descriptive analysis to determine how products were differentiated relative to one another. Consumer Just About Right (JAR) scores were evaluated by chi-squared analysis, and purchase intent was evaluated using a Kruskal-Wallis test with Dunn's post hoc test, using methods previously described by Jo et al. [18].

## 3. Results and Discussion

### 3.1. Feed Analysis

While all experimental diets were formulated to be isocaloric (3080 kcal/kg metabolizable energy) and isonitrogenous (18% crude protein), chemical analysis was conducted to determine the fatty acid profile of the experimental diets. There were no differences in the levels of crude total fat or gross energy between experimental feed samples (Table 2). In contrast, the control diet had significantly greater amounts of stearic acid and palmitic acid ( $p < 0.01$ ) levels in comparison to the other treatment groups (Table 2). Moreover, the control diet had the greatest percentage of total cholesterol in comparison to the other dietary treatment groups. The HOPN diet contained the highest percentage of oleic fatty acid content of all the diets, followed by the OA diet, with the control diet having the lowest oleic fatty acid content ( $p < 0.05$ ; Table 2).

### 3.2. Hen Performance

There were no mortalities over the 8-week feeding trial, and all laying hens were healthy and exhibited normal behavior. There were no significant treatment differences in body weights at the onset or termination of the 8-week feeding trial (Table 3). Interestingly, while there were no significant treatment differences in average egg weights at 4-weeks or 8 weeks of the study, the 8-week average egg weights produced from hens fed the HOPN dietary treatment were significantly smaller than the controls or OA group ( $p = 0.0004$ , Table 3). At 2 weeks and 6 weeks, the egg weights from hens fed the HOPN diets produced eggs less than egg weights from the control group, while egg weights were similar between HOPN and OA treatment groups (Table 3). This parallels results by Toomer et al. [10] and Van Elswyk et al. [19], demonstrating a reduction in egg mass when laying hens are fed diets rich in unsaturated fatty acids relative to conventionally produced eggs.

**Table 2.** Lipid content and fatty acid analysis of dietary treatments <sup>1</sup>.

Item Measured	Treatments			p-Value
	Control	HOPN	OA	
Crude Fat <sup>2</sup> (%)	10.0 ± 0.2	12.7 ± 0.2	6.2 ± 0.2	0.07
Palmitic Acid <sup>2</sup> (%)	22.6 ± 0.005 <sup>a</sup>	7.5 ± 0.005 <sup>c</sup>	14.7 ± 0.005 <sup>b</sup>	0.001
Stearic Acid <sup>2</sup> (%)	5.4 ± 0.002 <sup>a</sup>	1.9 ± 0.002 <sup>c</sup>	3.8 ± 0.002 <sup>b</sup>	0.001
Oleic Acid <sup>2</sup> %	38.4 ± 0.01 <sup>c</sup>	74.2 ± 0.01 <sup>a</sup>	43.8 ± 0.01 <sup>b</sup>	0.02
Trans-Elaidic <sup>2</sup> (%)	2.8 ± 0.02 <sup>b</sup>	0.9 ± 0.02 <sup>c</sup>	5.8 ± 0.02 <sup>a</sup>	0.01
Linoleic Acid <sup>2</sup> (%)	21.7 ± 0.002 <sup>a</sup>	8.8 ± 0.002 <sup>b</sup>	20.7 ± 0.002 <sup>a</sup>	0.03
Linolenic Acid <sup>2</sup> (%)	1.0 ± 0.002	0.4 ± 0.002	1.7 ± 0.002	0.07
Omega 3 <sup>2</sup> (%)	1.0 ± 0.001	0.4 ± 0.001	1.7 ± 0.001	0.07
Omega 6 <sup>2</sup> (%)	21.7 ± 0.001 <sup>a</sup>	8.8 ± 0.001 <sup>b</sup>	1.3 ± 0.001 <sup>c</sup>	0.03
β-Carotene (ppm)	<5.0	<5.0	<5.0	0.07
Total Cholesterol (mg/100 g)	50.4 ± 0.01 <sup>a</sup>	8.4 ± 0.01 <sup>b</sup>	3.6 ± 0.01 <sup>c</sup>	0.0
Gross Energy (kcal/kg)	4129 ± 0.06	4205 ± 0.06	3802 ± 0.06	0.07

<sup>1</sup> Treatments: control = conventional soybean meal and corn diet, HOPN = unblanched high-oleic peanut crumbles (20%) and corn diet, OA = control diet supplemented with 2.64% food-grade oleic fatty acid oil. Lipid (crude fat, total cholesterol), fatty acid, and beta-carotene analysis was performed by an AOAC-certified lab, ATC Scientific (Little Rock, AR, USA) food-grade oleic fatty acid oil. <sup>2</sup> Crude Fat content = g crude fat/g total sample weight \* 100, Fatty acid content = g of fatty acid/g total lipid content \* 100. Total cholesterol (mg/100 g dry weight) and lipid analysis was determined by direct methylation methods. Gross energy analysis was determined using an adiabatic oxygen bomb calorimeter and standard methods. Each value represents the mean ± the standard error for each triplicate sample. <sup>a,b,c</sup> Means within the same row with differing superscripts are significantly different ( $p < 0.05$ ).

**Table 3.** Performance of laying hens fed a diet of high-oleic peanuts or oleic acid.

Variable	Treatments <sup>1</sup>			p-Value
	Control	HOPN	OA	
Body Weight (g)				
Week 1	2059 ± 61.9	2009 ± 61.9	1989 ± 61.9	0.50
Week 8	2257 ± 53.5	2128 ± 53.5	2185 ± 53.5	0.06
Egg Weights (g) <sup>2</sup>				
Week 2	67.3 ± 1.4 <sup>a</sup>	62.4 ± 1.4 <sup>b</sup>	64.7 ± 1.4 <sup>ab</sup>	0.006
Week 4	66.6 ± 1.7	65.6 ± 1.7	66.3 ± 1.7	0.83
Week 6	66.6 ± 1.4 <sup>a</sup>	62.0 ± 1.4 <sup>b</sup>	65.3 ± 1.4 <sup>ab</sup>	0.005
Week 8	68.0 ± 1.7	65.7 ± 1.7	64.9 ± 1.7	0.18
8week Ave Egg Weight (g)	67.1 ± 0.6 <sup>a</sup>	63.9 ± 0.6 <sup>c</sup>	65.3 ± 0.6 <sup>b</sup>	0.0004
8 week FCR <sup>3</sup>	2.4±0.09	2.2±0.09	2.1±0.09	0.07

In total, 99 brown Leghorn (57 week of lay) hens were fed one of three isonitrogenous (18% crude protein) diets ad libitum for 8 weeks. Body weights were collected at week 1 and week 8 of the study (33 hens per treatment, 3 replicates, with 11 birds per replicate). <sup>1</sup> Treatments: control = conventional soybean meal and corn mash diet, HOPN = 20% unblanched high-oleic peanut + corn diet, OA = control diet supplemented with 2.64% food-grade oleic fatty acid oil. <sup>2</sup> Weights (g) of eggs were determined daily and weekly for each treatment group. Data represents the bi-weekly (2, 4, 6, and 8 weeks) averages ± standard error for each time point for each treatment group. <sup>3</sup> Feed conversion ratio (FCR) = kg and total feed intake over the 8-week/total dozen eggs produced over 8 weeks for each treatment group. Total number of eggs produced: Control = 1598 eggs, HOPN = 1617 eggs (≈49 eggs/hen), and OA = 1665 over the 8-week feeding trial; Each value (body weights and egg weights) represents the mean ± the standard error. <sup>a,b,c</sup> Means within the same row lacking a common superscript differ significantly ( $p < 0.05$ ).

Egg size has been shown to be greatly influenced by body weight and age [20], nutrition [21], and pullet management [22]. Egg size has been shown to be directly influenced by body weight; for every 45 g of body weight increase, there is a 0.5-g increase in egg size from 18 weeks of age in laying hens [22]. Hence, as the laying hen ages in the production cycle and increases in body weight, there is a proportionate increase in egg size [22]. The United States Department of Agriculture egg classification system [23–25] categorizes eggs by minimum weight per dozen eggs: jumbo 30 ounces (>63 g content per egg), extra-large 27 ounces (63–56 g content per egg), large 24 ounces (56–50 g content per egg), medium 21 ounces (50–44 g content per egg), small 18 ounces (44–38 g content per egg), peewee 15 ounces (<38 g content per egg) [25]. While there were no treatment differences in the total number of eggs produced, with 1598 eggs (≈48 eggs/hen) produced from the

control group, 1617 eggs ( $\approx 49$  eggs/hen) produced from the HOPN group, and 1665 eggs ( $\approx 50$  eggs/hen) produced from the OA group over the 8-week feeding trial, the control fed hens produced 1246 total jumbo eggs and 352 x-large size eggs, and the OA fed hens produced 1299 jumbo eggs and 366 x-large eggs, respectively. Conversely, hens fed the HOPN diet produced a total of 711 jumbo eggs and 906 x-large eggs over the 8-week feeding trial.

Therefore, in this study, jumbo eggs ( $\approx 63$  g content per egg) were the predominate egg classification for most eggs produced from older production hens (57 week of lay) in all treatment groups. In contrast to our previous high-oleic peanut layer feeding trial with younger age production hens (40 weeks of lay), extra-large eggs were the predominate egg classification for most eggs produced [10], which parallels other studies demonstrating a trend of increased egg size with hen production age [26]. Nonetheless, in this study, eggs produced from hens fed the HOPN diet had significantly smaller egg size/mass relative to the other treatment groups ( $p < 0.001$ ) at 57 weeks of lay, suggesting that a diet rich in unsaturated fats may be an effective commercial feeding regimen to manage the production of oversized eggs in older production hens.

While the feed conversion ratio (calculated as the total kg of feed intake over 8 weeks/total # dozen eggs produced over 8 weeks) of the control group (2.4 kg/dozen eggs) was higher than the other treatment groups (2.2 = HOPN, 2.1 = OA), there was no significant treatment differences between the feed conversion ratio (FCR) between the treatment groups (Table 3). FCR is a very important production parameter within the commercial egg industry, as a predictor of profitability utilizing the cost of kg of feed consumed [27] per total number of eggs produced. Thus, FCR is a measure of how efficiently an animal utilizes incoming dietary feed nutrients to generate the desired product of meat and/or eggs [27]. Typically, within the US commercial egg production industry, the typical FCR for each hen is about 2 kg of feed consumed/kg of egg produced, with each hen producing about 330 eggs per year [28].

### 3.3. Egg Grading, Quality, and Production

All eggs produced in this feeding trial were graded as USDA Grade AA of superior quality, with thick, firm egg whites and defect-free egg yolks. Additionally, the shells were clean and without defects. There were minimal numbers of blood spots or meat spots, with no statistical difference at the 95% confidence interval between eggs produced between the treatment groups (data not shown). The Haugh Unit (HU), first defined by Raymond Haugh [12], is commonly used to measure albumen (egg white) quality from the height and thickness of the albumen. Hence, fresher, higher-quality eggs have thicker egg whites and thus higher HU values. In this study, there were no significant differences in the average weekly HU between the treatment groups (Table 4). The vitelline membrane is a two-layer transparent casing enclosing the yolk separating the yolk from the egg albumen [29]. Vitelline membrane strength is an important physical attribute key to processing shell eggs and the separation of egg yolk from the albumen. Vitelline membrane strength is often used as a measurement of freshness since the vitelline membrane strength is time-dependent and reduced with increased time and storage handling conditions [29]. There were no significant differences in the average weekly shell strength or vitelline membrane strength between the treatment groups (Table 4).

However, the average weekly egg yolk color of eggs produced from hens fed the HOPN diet were significantly higher compared to eggs produced from hens fed the control and OA diets ( $p < 0.0001$ , Table 4). Moreover, upon visual observation, egg yolks from hens fed the HOPN diets were a visibly darker yellow/orange color intensity in comparison to the egg yolks produced from hens fed the control and OA diets (Figure 1).

**Table 4.** Egg quality of eggs produced from laying hens fed a diet of high-oleic peanuts or oleic acid.

Item	Treatments <sup>1</sup>			<i>p</i> -Value
	Control	HOPN	OA	
Shell Strength (g force) 8 week ave	4833.6 ± 101.8	5079.6 ± 100.5	4945.5 ± 100.5	0.23
Vitelline Membrane Strength (g force) 8 week ave	0.23 ± 0.004	0.24 ± 0.004	0.24 ± 0.004	0.36
Haugh Unit (HU) 8 week ave	92.7 ± 0.71	93.6 ± 0.70	92.9 ± 0.69	0.60
Yolk Color Roche (1–15) 8 week ave	4.9 ± 0.10 <sup>c</sup>	6.7 ± 0.10 <sup>a</sup>	5.4 ± 0.10 <sup>b</sup>	<0.0001

In total, 99 brown Leghorn (57 week of lay) hens were fed one of three isonitrogenous (18% crude protein) diets ad libitum for 8 weeks. Egg quality (Haugh unit, yolk color score, vitelline membrane strength, and shell strength) was determined bi-weekly (2, 4, 6, and 8 weeks) using the Technical Services and Supplies (TSS) QCD system, with calibration with the DSM Color Fan for yolk color. <sup>1</sup> Treatments: Control = conventional soybean meal and corn mash diet, HOPN = 20% unblanched high-oleic peanut + corn diet, OA = control diet supplemented with 2.64% food-grade oleic fatty acid oil. Eggs were collected weekly and analyzed for quality. Yolk color = Roche Color Fan color index 1–15 (lightest to darkest color intensity). Each value represents the 8-weekly average ± the standard error with 12 eggs/treatment (3 eggs/replicate), N = 36 total measured bi-weekly for 8 weeks. <sup>a,b,c</sup> Means on the same row lacking a common superscript differ significantly, (*p* < 0.05).



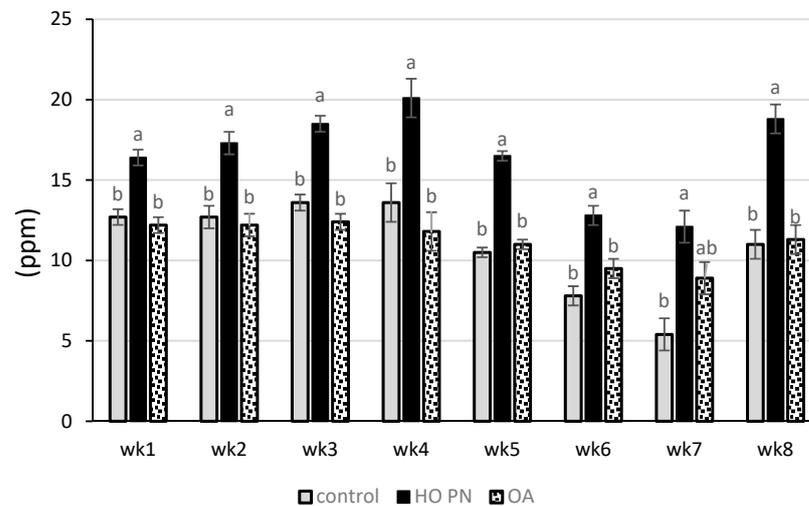
**Figure 1.** Representative images of yolk color within whole egg samples from each treatment group at week 8 of the feeding trial. Ninety-nine brown Leghorn (57 week of lay) hens were fed one of three isonitrogenous (18% crude protein) diets ad libitum for 8 weeks. At 8-weeks, one whole egg was randomly selected for this photograph as a representative of yolk color observations seen on the day of egg processing with 12 eggs per treatment. This image is not representative of any other egg quality parameters measured. Treatments: Control = conventional soybean meal and corn mash diet, HOPN = 20% unblanched high-oleic peanut + corn diet, OA = control diet supplemented with 2.64% food-grade oleic fatty acid oil.

Similarly, in the yolk color score, the  $\beta$ -carotene content in eggs produced from hens fed the HOPN diet was significantly greater than the  $\beta$ -carotene content in eggs produced from hens fed the control or OA diets at all time points measured (Figure 2; Week 1 *p* < 0.01, Week 2 *p* < 0.01, Week 3 *p* < 0.001, Week 4 *p* < 0.01, Week 5 *p* < 0.0001, Week 6 *p* < 0.01, Week 7 *p* < 0.01, Week 8 *p* < 0.01).  $\beta$ -carotene is a carotenoid, which is a lipid soluble antioxidant found abundantly in plants, is responsible for the rich yellow and deep orange colors in plants, and is a precursor to vitamin A [30]. Conventional commercial eggs are rich in lutein and zeaxanthin [31]. However, yolk lutein and zeaxanthin are highly subjective to oxidation during egg processing, storage, transport, and/or cooking [31].

There were no treatment differences in total cholesterol content between eggs produced from the three treatment groups over the course of the study (Table 5). Similarly, there were no significant treatment differences in total crude fat content in eggs produced from the three treatment groups at weeks 2, 4, 5, 6, 7, or 8 (Table 5). But at week 3 of the 8-week feeding trial, eggs produced from hens fed the OA diet had significantly reduced levels of crude fat relative to the other treatments (*p* < 0.01, Table 5). At week 2 and week 5, there were no significant treatment differences in palmitic acid content between eggs

produced from hens fed the three treatment groups (Table 5). At week 3, 4, 6, 7, and 8, eggs produced from hens fed the HOPN diet had significantly less palmitic acid levels in comparison to eggs produced from the other treatment groups (Table 5). While there were no significant treatment differences in stearic acid levels in eggs produced from the three treatment groups at week 2, eggs produced from hens fed the HOPN and OA diets had significantly lower levels of stearic acid, relative to eggs produced from the control group at experimental weeks 3, 4, 6, 7, and 8 of the study (Table 5). Stearic acid content was similar between eggs produced from hens fed the HOPN and OA diets at weeks 3, 4, 6, 7, and 8 (Table 5). Stearic acid content was lowest in eggs produced from laying hens fed the HOPN treatment group relative to the other treatments only at week 5 of the study ( $p < 0.05$ ).

Monounsaturated OA content was similar between eggs produced from the three treatment groups at week 2 (Table 5). OA content was significantly different between eggs produced from each of the treatment groups, with the highest oleic acid content in eggs produced from hens fed the HOPN diet and lowest in control eggs at week 3 ( $p < 0.0001$ ), week 4 ( $p < 0.0001$ ), week 6 ( $p < 0.001$ ), week 7 ( $p < 0.0001$ ), and week 8 ( $p < 0.0001$ ). At week 5, OA content was significantly greater in eggs produced from hens fed the HOPN diet, while OA levels were similar between eggs produced from hens fed the control and OA diets ( $p < 0.01$ , Table 5). Total linoleic acid content was significantly greater in eggs produced from control fed hens at all time points (exception of week 4) relative to eggs produced from hens fed the HOPN diet, while levels were similar between eggs produced from hens fed the HOPN and OA diets at week 2, 5, 6, 7, and 8 (Table 6). Regardless, there were no significant treatment differences between the total linoleic content in eggs produced from hens fed the three treatment groups at week 4 only (Table 6).



**Figure 2.** Effect of diet on the  $\beta$ -Carotene content of eggs produced. In total, 99 brown Leghorn (57 weeks of lay) hens were fed one of three isonitrogenous (18% crude protein) diets ad libitum for 8 weeks. Treatments: Control = conventional soybean meal and corn mash diet, HOPN = 20% unblanched high-oleic peanut + corn diet, OA = control diet supplemented with 2.64% food-grade oleic fatty acid oil. Beta-carotene analysis was performed by an AOAC-certified lab, ATC Scientific (Little Rock, AR, USA).  $\beta$ -carotene content in eggs was determined using AOAC 958.05 methods [14].  $p$ -values at the various sampling time-points were the following: Week-1  $p = 0.002$ , Week-2  $p = 0.004$ , Week-3  $p = 0.0003$ , Week-4  $p = 0.006$ , Week-5  $p < 0.0001$ , Week-6  $p = 0.004$ , Week-7  $p = 0.008$ , Week-8  $p = 0.001$ . Each bar column represents the average  $\pm$  the standard error for each experimental time-point, with 15 egg samples/treatment group (5 egg samples per replicate, 3 replicates) and a total of 45 eggs analyzed at each time-point. <sup>a,b</sup> Bar columns with differing superscript are significantly different ( $p < 0.05$ ).

**Table 5.** The effect of feeding laying hens a high-oleic peanut (HOPN) diet or an oleic fatty acid (OA) diet on the fatty acid profile of the eggs produced.

Week	Item Measured	Treatments <sup>1</sup>			p-Value
		Control	HOPN	OA	
2	Cholesterol (mg/100 g)	310.1 ± 16.3	312.0 ± 16.3	309.6 ± 16.3	0.99
	Crude Fat (%)	9.0 ± 1.0	7.5 ± 1.0	6.0 ± 1.0	0.19
	Palmitic (%)	2.0 ± 0.21	1.4 ± 0.21	1.3 ± 0.21	0.09
	Stearic (%)	0.7 ± 0.07	0.5 ± 0.07	0.4 ± 0.07	0.05
	Oleic (%)	3.9 ± 0.5	4.1 ± 0.5	2.7 ± 0.5	0.15
3	Cholesterol (mg/100 g)	318.7 ± 16.7	327.0 ± 16.7	279.5 ± 16.7	0.18
	Crude Fat (%)	10.0 ± 0.5 <sup>a</sup>	10.3 ± 0.5 <sup>a</sup>	6.2 ± 0.5 <sup>b</sup>	0.003
	Palmitic (%)	21.9 ± 0.2 <sup>a</sup>	18.7 ± 0.2 <sup>b</sup>	21.5 ± 0.2 <sup>a</sup>	0.0002
	Stearic (%)	7.6 ± 0.2 <sup>a</sup>	6.6 ± 0.2 <sup>b</sup>	6.8 ± 0.2 <sup>b</sup>	0.015
	Oleic (%)	42.4 ± 0.1 <sup>c</sup>	53.2 ± 0.1 <sup>a</sup>	46.0 ± 0.1 <sup>b</sup>	<0.0001
4	Cholesterol (mg/100 g)	330.2 ± 19.1	334.7 ± 19.1	294.9 ± 19.1	0.34
	Crude Fat (%)	8.2 ± 0.9	8.1 ± 0.9	8.1 ± 0.9	0.99
	Palmitic (%)	21.5 ± 0.2 <sup>a</sup>	18.3 ± 0.2 <sup>b</sup>	21.8 ± 0.2 <sup>a</sup>	<0.0001
	Stearic (%)	7.4 ± 0.1 <sup>a</sup>	6.4 ± 0.1 <sup>b</sup>	6.8 ± 0.1 <sup>b</sup>	0.002
	Oleic (%)	41.5 ± 0.4 <sup>c</sup>	53.9 ± 0.4 <sup>a</sup>	47.2 ± 0.4 <sup>b</sup>	<0.0001
5	Cholesterol (mg/100 g)	330.9 ± 9.5	325.6 ± 9.5	301.2 ± 9.5	0.14
	Crude Fat (%)	8.7 ± 1.0	7.5 ± 1.0	7.4 ± 1.0	0.64
	Palmitic (%)	20.7 ± 0.8	18.1 ± 0.8	20.4 ± 0.8	0.12
	Stearic (%)	7.2 ± 0.2 <sup>a</sup>	6.2 ± 0.2 <sup>b</sup>	7.2 ± 0.1 <sup>a</sup>	0.03
	Oleic (%)	41.4 ± 1.5 <sup>b</sup>	53.7 ± 1.5 <sup>a</sup>	44.7 ± 1.5 <sup>b</sup>	0.003
6	Cholesterol (mg/100 g)	307.6 ± 16.6	312.8 ± 16.6	311.6 ± 16.6	0.97
	Crude Fat (%)	5.8 ± 0.6	6.2 ± 0.6	6.3 ± 0.6	0.81
	Palmitic (%)	20.2 ± 0.21 <sup>a</sup>	17.9 ± 0.21 <sup>b</sup>	21.1 ± 0.21 <sup>a</sup>	0.01
	Stearic (%)	7.1 ± 0.1 <sup>a</sup>	6.5 ± 0.1 <sup>b</sup>	6.4 ± 0.1 <sup>b</sup>	0.007
	Oleic (%)	39.8 ± 0.9 <sup>c</sup>	52.3 ± 0.9 <sup>a</sup>	44.8 ± 0.9 <sup>b</sup>	0.0002
7	Cholesterol (mg/100 g)	305.9 ± 8.5	280.1 ± 8.5	312.9 ± 8.5	0.08
	Crude Fat (%)	6.3 ± 0.5	6.5 ± 0.5	6.1 ± 0.5	0.82
	Palmitic (%)	22.1 ± 0.2 <sup>a</sup>	18.8 ± 0.2 <sup>b</sup>	22.1 ± 0.2 <sup>a</sup>	<0.0001
	Stearic (%)	7.8 ± 0.2 <sup>a</sup>	6.4 ± 0.2 <sup>b</sup>	6.9 ± 0.2 <sup>b</sup>	0.003
	Oleic (%)	43.4 ± 0.4 <sup>c</sup>	54.5 ± 0.4 <sup>a</sup>	48.2 ± 0.4 <sup>b</sup>	<0.0001
8	Cholesterol (mg/100 g)	327.5 ± 13.2	351.2 ± 13.2	330.0 ± 13.2	0.43
	Crude Fat (%)	7.8 ± 0.7	6.6 ± 0.7	5.2 ± 0.7	0.12
	Palmitic (%)	21.6 ± 0.3 <sup>a</sup>	18.2 ± 0.3 <sup>b</sup>	21.7 ± 0.3 <sup>a</sup>	0.0003
	Stearic (%)	7.7 ± 0.1 <sup>a</sup>	6.4 ± 0.1 <sup>b</sup>	6.6 ± 0.1 <sup>b</sup>	0.0002
	Oleic (%)	42.2 ± 0.5 <sup>c</sup>	51.6 ± 0.5 <sup>a</sup>	46.7 ± 0.5 <sup>b</sup>	<0.0001

In total, 99 brown Leghorn (57 week of lay) hens were fed one of three isonitrogenous (18% crude protein) diets ad libitum for 8 weeks. Total cholesterol (mg/100 g weight sample) and lipid analysis was determined by direct methylation methods. Egg fat hydrolysis methods were measured using the AOAC method 954.02. <sup>1</sup> Treatments: Control = conventional soybean meal and corn mash diet, HOPN = 20% unblanched high-oleic peanut + corn diet, OA = control diet supplemented with 2.64% food-grade oleic fatty acid oil. Crude fat content = g crude fat/g total sample weight \* 100, fatty acid content = g of fatty acid/g total lipid content \* 100. Each value represents the mean ± the standard error for each experimental time-point with 15 egg samples/treatment group (5 egg samples per replicate, 3 replicates). In total, 45 eggs were analyzed at each time-point. <sup>a,b,c</sup> Means within the same row lacking a common superscript differ significantly ( $p < 0.05$ ).

There were no significant differences in the total linolenic fatty acid content in eggs produced from hens fed the three treatment groups at week 2 (Table 6). Total linolenic fatty acid content was significantly lower in eggs produced from hens fed the HOPN diet in comparison to the total linolenic fatty acid content in eggs produced from hens fed the control and OA treatments at week 3 ( $p < 0.0001$ ), week 4 ( $p < 0.001$ ), week 5 ( $p < 0.01$ ), week 7 ( $p < 0.001$ ), and week 8 ( $p < 0.001$ ). The total linolenic fatty acid content was similar between eggs in the control and OA treatment groups at week 3, 4, 5, and 6. Even so, the average total linolenic acid content in all egg samples from all treatment groups within the study were very low ( $\leq 0.2\%$ , Table 6).

**Table 6.** The effect of feeding laying hens a high-oleic peanut (HOPN) diet or an oleic fatty acid (OA) diet on the unsaturated fatty acid profile of the eggs produced.

Week	Item (%)	Treatments <sup>1</sup>			<i>p</i> -Value
		Control	HOPN	OA	
2	Linoleic	1.1 ± 0.12 <sup>a</sup>	0.52 ± 0.12 <sup>b</sup>	0.56 ± 0.12 <sup>b</sup>	0.03
	Linolenic	0.02 ± 0.003	0.003 ± 0.003	0.003 ± 0.003	0.05
	Omega 3	0.17 ± 0.1	0.25 ± 0.1	0.29 ± 0.1	0.68
	Omega 6	1.3 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.05
	C22:6 n3 <sup>¥</sup>	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.63
3	Linoleic	11.4 ± 0.2 <sup>a</sup>	6.8 ± 0.2 <sup>c</sup>	8.6 ± 0.2 <sup>b</sup>	<0.0001
	Linolenic	0.20 ± 0.003 <sup>a</sup>	0.10 ± 0.003 <sup>b</sup>	0.20 ± 0.003 <sup>a</sup>	<0.0001
	Omega 3	3.4 ± 0.3	4.3 ± 0.3	4.3 ± 0.3	0.15
	Omega 6	13.5 ± 0.2 <sup>a</sup>	9.0 ± 0.2 <sup>c</sup>	10.7 ± 0.2 <sup>b</sup>	<0.0001
	C22:6 n3 <sup>¥</sup>	3.2 ± 0.3	4.2 ± 0.3	4.2 ± 0.3	0.11
4	Linoleic	7.6 ± 2.2	6.6 ± 2.2	8.2 ± 2.2	0.88
	Linolenic	0.20 ± 0.009 <sup>a</sup>	0.10 ± 0.009 <sup>b</sup>	0.20 ± 0.009 <sup>a</sup>	0.0004
	Omega3	4.9 ± 0.3	4.3 ± 0.3	4.1 ± 0.3	0.26
	Omega 6	9.7 ± 2.2	8.8 ± 2.2	10.2 ± 2.2	0.90
	C22:6 n3 <sup>¥</sup>	4.7 ± 0.3	4.2 ± 0.3	3.9 ± 0.3	0.31
5	Linoleic	9.6 ± 0.4 <sup>a</sup>	6.6 ± 0.4 <sup>b</sup>	7.5 ± 0.4 <sup>b</sup>	0.004
	Linolenic	0.17 ± 0.01 <sup>a</sup>	0.09 ± 0.01 <sup>b</sup>	0.14 ± 0.01 <sup>a</sup>	0.006
	Omega 3	6.6 ± 1.6	5.1 ± 1.6	6.9 ± 1.6	0.72
	Omega 6	11.5 ± 0.4 <sup>a</sup>	8.6 ± 0.4 <sup>b</sup>	9.3 ± 0.4 <sup>b</sup>	0.006
	C22:6 n3 <sup>¥</sup>	6.4 ± 1.67	5.0 ± 1.6	6.8 ± 1.6	0.73
6	Linoleic	9.1 ± 0.4 <sup>a</sup>	6.7 ± 0.4 <sup>b</sup>	7.7 ± 0.4 <sup>ab</sup>	0.008
	Linolenic	0.16 ± 0.01 <sup>a</sup>	0.10 ± 0.01 <sup>b</sup>	0.12 ± 0.01 <sup>ab</sup>	0.02
	Omega 3	8.0 ± 1.0	5.6 ± 1.0	6.3 ± 1.0	0.30
	Omega 6	11.1 ± 0.4 <sup>a</sup>	8.7 ± 0.4 <sup>b</sup>	9.8 ± 0.4 <sup>ab</sup>	0.02
	C22:6 n3 <sup>¥</sup>	7.8 ± 1.0	5.5 ± 1.0	6.1 ± 1.0	0.31
7	Linoleic	10.0 ± 1.1 <sup>a</sup>	4.8 ± 1.1 <sup>b</sup>	7.9 ± 1.1 <sup>ab</sup>	0.04
	Linolenic	0.20 ± 0.006 <sup>a</sup>	0.10 ± 0.006 <sup>c</sup>	0.13 ± 0.006 <sup>b</sup>	0.0002
	Omega 3	4.1 ± 0.4	3.8 ± 0.4	3.3 ± 0.4	0.38
	Omega 6	12.0 ± 1.1	6.9 ± 1.1	10.0 ± 1.1	0.05
	C22:6 n3 <sup>¥</sup>	3.9 ± 0.4	3.8 ± 0.4	3.2 ± 0.3	0.41
8	Linoleic	10.5 ± 0.3 <sup>a</sup>	7.2 ± 0.3 <sup>b</sup>	7.6 ± 0.3 <sup>b</sup>	0.0002
	Linolenic	0.18 ± 0.007 <sup>a</sup>	0.09 ± 0.007 <sup>c</sup>	0.13 ± 0.007 <sup>b</sup>	0.0004
	Omega 3	4.7 ± 0.4	5.6 ± 0.4	4.4 ± 0.4	0.22
	Omega 6	12.7 ± 0.3 <sup>a</sup>	9.7 ± 0.3 <sup>b</sup>	9.6 ± 0.3 <sup>b</sup>	0.0002
	C22:6 n3 <sup>¥</sup>	4.5 ± 0.4	5.5 ± 0.4	4.3 ± 0.4	0.19

In total, 99 brown Leghorn (57 week of lay) hens were fed one of three isonitrogenous (18% crude protein) diets ad libitum for 8 weeks. Fatty acid analysis of egg samples was determined using standard direct methylation methods. Egg fat hydrolysis methods were measured using the AOAC method 954.02. <sup>1</sup> Treatments: Control = conventional soybean meal and corn mash diet, HOPN = 20% unblanched high-oleic peanut + corn diet, OA = control diet supplemented with 2.64% food-grade oleic fatty acid oil. Fatty acid content = g of fatty acid/g total lipid \* 100. Each value represents the mean ± the standard error for each experimental time-point with 15 egg samples/treatment group (5 egg samples per replicate, 3 replicates). In total, 45 were eggs analyzed at each time-point. <sup>¥</sup> C22:6 n3 = polyunsaturated docosahexaenoic fatty acid. <sup>a,b,c</sup> Means the same row lacking a common superscript differ significantly (*p* < 0.05).

There were no significant treatment differences in the total omega 3 content in eggs produced from hens from the three treatment groups at week 2, 3, 4, 5, 6, 7, or 8 (Table 6). There were no significant treatment differences in the total omega 6 content in eggs produced from the three treatment groups at week 2, week 4, or week 7 (Table 6). At week 3, eggs produced from hens fed the control diet had the highest content of total omega 6 fatty acid (*p* < 0.001), while eggs produced from hens fed the OA diet had an intermediate level of total omega 6 fatty acid, and eggs produced from hens fed the HOPN diet had the lowest omega 6 content in comparison to the other treatment groups (Table 6). At week 5, 6, and 8, eggs produced from hens fed the control diet had significantly higher levels of total omega 6 content in comparison to eggs produced from hens fed the HOPN

diet. Nonetheless, there were no significant differences in n3 docosahexaenoic (C22:6 n3) acid content in eggs produced from hens fed the three treatment groups at any of the experimental time points (Table 6). Additionally, eggs were analyzed for the following fatty acid acids: butyric, caproic, caprylic, undecanoic, lauric, tridecanoic, myristic, myristoleic, pentadecylic, pentadecenoic, margaric, margaroleic, arachidic, gadoleic, eicosadienoic, homo-gamma-linolenic, eicosatrienoic, arachidonic, n3 timnodonic, heneicosanic, behenic, erucic, brassic, lignoceric, and nervonic acid, of which no levels were detected (data not shown).

Numerous feeding trials have demonstrated that modification of the fatty acid profile in the diets of food production animals significantly alters the lipid content and fatty acid profile of the meat [32–34] and/or eggs produced [10,35–39]. Similarly, this study demonstrates that eggs produced from older production hens (57 weeks of lay) fed a HOPN or OA diet had significantly reduced saturated fatty acid and trans-fat content with enhanced monounsaturated oleic fatty acid content as compared to conventional eggs.

### 3.4. Sensory Evaluation

Of the 109-consumer panelists, 60% were female and 34% were male (data not shown). All consumers were under the age of 65, with 50% of the consumer population between the age of 18 to 25 (data not shown). A total of 37% of the consumer population reported the consumption of scrambled eggs multiple times per week, and 34% reported consuming scrambled eggs at least once per week (data not shown). Recruited consumer panelists scored scrambled eggs similarly in appearance liking, aroma liking, color liking, overall liking, flavor liking, texture Just About Right (JAR), and purchase intent between the three treatment groups ( $p < 0.05$ , Table 7) using a 9-point hedonic scale: extremely dislike = 1 and extremely like = 9.

**Table 7.** Consumer <sup>1</sup> acceptance scores for scrambled egg samples produced from laying hens fed experimental diets <sup>2</sup>.

	Control	OA	HOPN
Appearance Liking <sup>3</sup>	6.6 <sup>a</sup>	6.2 <sup>b</sup>	6.2 <sup>b</sup>
Aroma Liking <sup>3</sup>	6.6 <sup>a</sup>	6.5 <sup>a</sup>	6.4 <sup>a</sup>
Color Liking <sup>3</sup>	6.6 <sup>a</sup>	6.3 <sup>a</sup>	6.5 <sup>a</sup>
Overall Liking <sup>3</sup>	7.0 <sup>a</sup>	6.6 <sup>a</sup>	6.6 <sup>a</sup>
Flavor Liking <sup>3</sup>	6.9 <sup>a</sup>	6.6 <sup>a</sup>	6.6 <sup>a</sup>
Overall Flavor JAR <sup>4</sup>	Not Enough Flavor	20.2% <sup>a</sup>	18.3% <sup>a</sup>
	JAR	66.1% <sup>a</sup>	65.1% <sup>a</sup>
	Too Much Flavor	13.8% <sup>a</sup>	16.5% <sup>a</sup>
Aftertaste JAR <sup>5</sup>	Not Strong Enough	10.0% <sup>a</sup>	6.0% <sup>a</sup>
	JAR	60.0% <sup>a</sup>	50.0% <sup>a</sup>
	Too Strong	30.0% <sup>a</sup>	44.0% <sup>a</sup>
Texture JAR <sup>4</sup>	Much Too Soft	12.8% <sup>a</sup>	4.6% <sup>a</sup>
	JAR	73.4 <sup>a</sup>	65.1% <sup>a</sup>
Purchase Intent <sup>6</sup>	3.7 <sup>a</sup>	3.5 <sup>a</sup>	3.4 <sup>a</sup>

<sup>1</sup> In total, 109 consumer panelists scored scrambled shell egg samples for comparative sensory attributes. <sup>2</sup> Experimental diets: Control = soybean meal + corn, OA = control diet spiked with 2.64% oleic fatty acid oil, HOPN = high-oleic peanut + corn. <sup>3</sup> Liking attributes scores: 1 = extremely dislike and 9 = extremely like. <sup>4</sup> Just About Right (JAR) scores: 1 or 2 = too little, 3 = just about right, and 4 or 5 = too much. The reported percentage of consumers that selected each option used Chi-square for statistical analysis. <sup>5</sup> Aftertaste liking and JAR scores were from consumers who detected an aftertaste in the product. <sup>6</sup> Purchase intent scores: 1 or 2 = would not buy, 3 = unsure, 4 or 5 = would buy. <sup>a,b</sup> Means within the same row lacking a common superscript differ significantly ( $p < 0.05$ ).

Food flavor is consistently rated as one of the most important elements determining consumer product consumption, timeframe on the food market, purchase intent, and repeat purchase [40,41]. Other studies have reported that eggs produced from hens fed diets supplemented with unsaturated fatty acids from marine oils or linseed oils have undesirable off-flavors in the eggs [42] and/or meat [42] produced. In contrast, this study demonstrated that consumer panelists equally scored and preferred scrambled eggs produced from hens fed diets containing unsaturated fatty acids from high-oleic peanuts or

oleic acid, with no reports of conceived off-flavors. Thus, in this study, we aimed to not only examine the effects of feeding production hens HOPN on the fatty acid profile of the shell eggs produced, but also to determine the effect on sensory attributes and consumer acceptance of the eggs produced. Hence, feeding a high-oleic peanut diet and/or OA supplemented diet did not adversely affect the sensory attributes or consumer acceptance of the eggs produced.

#### 4. Conclusions

Lastly, this study helps to substantiate the utilization of whole unblanched high-oleic peanuts as a valued alternative feed ingredient for poultry to enhance the eggs produced with  $\beta$ -carotene and reduced saturated fatty acid content. Furthermore, this study also confirms the use of high-oleic peanuts with the skin intact as an energy- and protein-rich alternative feed ingredient for older production hens to prevent the production of oversized eggs, while naturally enhancing yolk pigments and carotenoid content.

**Author Contributions:** All authors actively contributed to the care and husbandry of all research animals, while co-authors O.T.T., R.M., and K.E.A. were active participants in the data analysis, data interpretation, and preparation of the manuscript. Co-authors T.C.V., and E.S. collected and tabulated all body weights, feed intake, egg weights and egg quality data. Co-author A.K.R. additionally assisted with formatting and editing the final manuscript for publication. All authors have read and agreed to the published version of the manuscript.

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**Institutional Review Board Statement:** The procedures used in these studies were approved by the North Carolina State University Institutional Animal Care and Use Committee (IACUC #17-001-A).

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study. The sensory protocol was reviewed and deemed exempt by the NC State University Institutional Review Board (IRB) for human subjects.

**Data Availability Statement:** The data presented in this study are available on request from the corresponding author.

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**Conflicts of Interest:** There is absolutely no conflict of interest regarding this manuscript.

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