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Vitamins A, E and fatty acid composition of the eggs of caged hens and pastured hens

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Abstract

In the US farmers often market pastured poultry eggs for a premium price, claiming animal and human health benefits. We examined how moving pastured hens to forage legumes or mixed grasses influenced hen (*Gallus gallus* L.) egg omega-3 fatty acids and concentrations of vitamins A and E. We also compared the eggs of the pastured hens to those of hens fed a commercial diet in cages. We used a cross-over design to compare pasture species: 75 sister hens were assigned to one of three pasture treatment groups: (1) alfalfa (*Medicago sativa* L.), (2) red and white clover (*Trifolium pretense* L. and *Trifolium repens* L.) or (3) mixed cool season grasses. Groups were rotated to all three pasture treatments, each for 2 weeks and supplemented with 70 g commercial hen mash bird^{-1} day^{-1}. Pasture botanical composition, forage mass, leaf to total ratio and plant fatty acid composition were compared among pasture treatments. Eggs of the pastured hens were compared to eggs of 50 sister hens that were fed only commercial hen mash in cages for the entire 6 weeks. Forage parameters varied somewhat, but did not explain plant linolenic acid variation. Seventeen of the 18 quantified egg fatty acids, and vitamin A concentrations did not ($P < 0.05$) differ among the three pasture treatment groups. Eggs of the hens that foraged grasses had 23% more ($P < 0.0001$) vitamin E than eggs of hens that foraged clover. Compared to eggs of the caged hens, pastured hens' eggs had twice as much vitamin E and long-chain omega-3 fats, 2.5-fold more total omega-3 fatty acids, and less than half the ratio of omega-6:omega-3 fatty acids ($P < 0.0001$). Vitamin A concentration was 38% higher ($P < 0.05$) in the pastured hens' eggs than in the caged hens' eggs, but total vitamin A per egg did not differ. At the end of the experiment, pastured hens weighed 14% less and averaged 15% lower hen-day egg production than caged birds ($P < 0.0001$). Results suggest that grass pastures may enhance vitamin E in eggs of pastured hens more than clover, and pastured hens supplemented with commercial mash will produce eggs with significantly more vitamin E and total omega-3 fatty acids compared to eggs from caged hens fed only commercial hen mash. Pastured hens may have lower body weight and egg production than caged hens, unless they are supplemented adequately to meet their dietary energy and crude protein needs.

Key words: omega-3 fat, pastured poultry, vitamin A, vitamin E, poultry eggs, pasture, legumes

Introduction

Producing poultry on pasture or cover crops is becoming a popular way for livestock and crop farmers to diversify their operations in the United States[1]. Poultry are often rotated onto pastures after cattle or sheep, where they forage on regrowth and scavenge for invertebrates in manure deposits often helping to distribute manure nutrients. Recent research indicates that livestock products from animals that forage grasslands have a higher concentration of omega-3 fatty acids and fat-soluble vitamins than livestock products from animals that are fed grain and stored feed diets[2–5].

The chicken has a short digestive tract and can rapidly assimilate dietary nutrients. Fat-soluble vitamins in the diet are readily transferred to the liver and then the egg yolk. Naber[6] classified nutrients in the egg by responsiveness to dietary change and determined that all the fat-soluble vitamins, including A and E, and the unsaturated fats, linoleic and linolenic acids, were egg responsive and that hen diet had a marked influence on the egg concentration. Egg yolk vitamin A levels were significantly increased, in a

study from 0 to 32 weeks, at the first measure at 4 weeks, versus hens fed an unsupplemented control diet[7]. Egg yolk vitamin B_{12} levels measured weekly following dietary supplementation showed significant increases in yolk concentration within 1 week[8].

Studies of pastured poultry, however, are limited. In the UK Tolan et al.[9] compared the chemical composition of eggs produced by hens at six different research and educational institutions, in either: (1) cages, (2) floor pens, or (3) with access to grass, supplemented with a grain ration. Although the researchers did not compare the omega-3 fatty acid concentration of the eggs, the hens given access to grass tended to have more retinol than the eggs of the other hens, although differences were not significant at the $P < 0.01$ level used to test for significance.

In Spain, Lopez-Bote et al.[10] compared the diet and eggs of hens fed a commercial mixed feed for laying hens in Spain, to the eggs of 'free-range' hens. The free-range hens were given access to natural grassland, dominated by Italian ryegrass (*Lolium perenne* L.), and were fed 50 g hen^{-1} day^{-1} of the commercial hen feed. The grass compared to the commercial feed mix had 13-fold more α-tocopherol (vitamin E), 20-fold more linolenic acid (an omega-3 fatty acid) and 2.66-fold less linoleic acid (an omega-6 fatty acid). After 28 days on the two different dietary treatments (e.g. grass + commercial feed versus commercial feed), the egg yolks of the free-range hens had 30% more α-tocopherol ($P < 0.01$) and almost three-fold more ($P < 0.001$) omega-3 fatty acid than the eggs of the commercially fed hens. The ratio of omega-6 to omega-3 was also significantly lower ($P < 0.001$) in the pastured hens compared to those fed only commercial feed[10].

Research has shown that plants have the highest concentrations of unsaturated fats when they have a high leaf to stem ratio compared to stages of development when plants have a high proportion of stem tissue[3]. We have also found that vegetative legumes had more linolenic acid than grass species[11]. Since hen eggs are highly responsive to dietary changes of vitamins A and E, and the unsaturated fats, linoleic and linolenic acids[6], and because pastured hens can be rotated to species-specific pastures, we evaluated whether moving pastured hens to forage grass or common legume (alfalfa, red and white clover) pastures would influence hen egg composition. And because pastured poultry may have nutritional benefits that could enhance market value, we wanted to compare the eggs of pastured and caged birds to assess whether legume or grass pastures influenced egg composition.

Materials and Methods

Pastures

The experiment was conducted at the Pennsylvania State University Dairy Cattle Research and Education Center in State College, Pennsylvania (40°50′N, 77°51′W, elevation 352 m) from 1 July through 11 August 2002. The predominant soil series was a Hagerstown silty clay loam (fine, mixed, mesic Typic Hapludalfs, 3–8% slope). Prior to the experiment, the field was rotationally grazed with dairy heifers and dry cows, with excess forage harvested for haylage. Pastures were mowed occasionally to maintain them in similar vegetative stages of development. Lime, phosphorus and potassium were applied to the experimental area according to soil test recommendations to achieve optimum levels for forage production.

Three pastures were evaluated in this experiment: (1) alfalfa: 'Alfagraze' alfalfa with minor contributions of 'Pennlate' orchardgrass (*Dactylis glomerata* L.), 'Bull' tall fescue (*Festuca arundinacea* Schreb) and 'Saratoga' smooth bromegrass (*Bromus inermis* Leyss.); (2) red and white clover: 'Cinnamon' red clover and 'California Ladino' white clover with minor contributions of 'Pennlate' orchardgrass, 'Bull' tall fescue and 'Saratoga' smooth bromegrass; and (3) mixed cool-season grass pasture dominated by naturalized ecotypes of cool-season grasses that had been planted about 30 years prior to the experiment. The dominant species were orchardgrass, and smooth bromegrass, with some quackgrass, Kentucky bluegrass (*Poa pretense* L.), timothy (*Phleum pratense* L.) and tall fescue.

Experimental design

We compared the effect of moving foraging hens among three different pasture types on the hens' eggs, using a cross-over design[12]. Three groups of 25 pastured hens foraged on one of three pasture treatments for 14 days. The hens were then rotated to the other two pasture treatments, each for 14 days. The eggs of the three groups of chickens were collected for comparison on the last 3 days of each 14-day treatment period (days 12, 13 and 14). To compare the eggs of pastured hens to caged hens fed a commercial hen mash, we used a two-way design. Another group of 50 sister hens in cages was maintained under commercial conditions over the same 6-week experimental period. Caged hens' eggs were collected to compare to the pastured hens' eggs at the end of the first and last (the third) feeding periods. We compared the three pasture feeding treatments and the caged feeding treatments with a pre-planned contrast (pastured versus caged hens).

Animal management

Four weeks prior to putting the birds on pasture, the 75 pasture treatment pullets were offered supplemental alfalfa hay *ad libitum* (12 weeks) in floor pens indoors. Hy-Line variety brown egg laying pullets (age 12 weeks) were stratified by weight; 50 birds were assigned to commercial cages (387 cm^2 bird^{-1}) in an environmentally controlled room at 23°C. Caged birds were fed commercial hen mash [see Table 1, 16.9% crude protein (CP), 2863 kcal kg^{-1} metabolizable energy (ME)] and water *ad libitum* for the duration of the trial, to reflect management practices of a commercial confinement operation.

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Table 1. Commercial hen mash composition.

	(kg T^{-1})
Bakery by-product meal	20
Canola meal	50
Corn	551.64
Distillers dried grain solubles	60
Dicalcium phosphate	1.50
Calcium chips ($CaCO_3$)	53.5
Poultry meal	80
Salt	2.5
Soybean meal	98.5
Limestone	39.5
Wheat middlings	29
Fat (animal and vegetable blend)	6
Methionine (liquid)	4
Lysine	1.65
Threonine	0.3
Enzymes (Avizyme® 0.75, phytase 0.06)	0.4
Vitamin premix	0.5
Trace mineral premix	0.5
Choline chloride	0.5

Three weeks prior to the beginning of the experimental trial (16 weeks), the pasture treatment pullets were acclimated to a pasture adjacent to the experimental area. On day 0 of the experiment (1 July), the pasture feed treatment hens were randomly assigned and given access to one of three pasture mixtures. Each group of 25 birds was then rotated to a different forage treatment every 14 days until all three hen groups had grazed all three forage treatments (42 days total, 11 August). Pastured hens received 70 g hen^{-1} day^{-1} of commercial hen mash (35 g hen^{-1} at 12.00 and at 20.00 hours), and calcium carbonate chips and water *ad libitum*. The birds were given access to the pasture treatment that they were assigned to from 08.00 to 20.00 hours daily. At 20.00 hours, pastured hens were confined to mobile coops (2.44 × 3.05 m) for the night for protection from predators and to encourage egg-laying in coop nests.

The hen coops were on wheels, and hens were moved to a new paddock every 3 days to maintain fresh forage for the hens. Each coop contained three colony nests, a 45.7 cm diameter pan feeder, and a nipple drinker system sufficient for 25 birds. An electrified polywire poultry fence (14 horizontal wires, 1.2 m height, 889 mm semi-rigid stay spacing, 49.8 m linear perimeter; Kencove Farm Fence Supplies, Blairsville, Pennsylvania, USA) was used to create temporary paddocks of 6.9×10^{-3} ha area to restrict them to their assigned pasture treatment and for predator protection for the birds during daylight hours.

Forage sampling methods

Plant tissue for fatty acid analysis. On day 6 of each 2-week treatment period, immediately prior to hens foraging, a 'hand-plucked' sample that mimicked the foraging action of the birds, was collected from four random locations in each pasture treatment. Approximately 20 g dry matter (DM) was collected for fatty acid analysis. Plant tissue samples were immediately frozen in liquid N, freeze-dried, returned to the laboratory, ground to pass a 1 mm screen, and stored in a −80°C freezer until they were analyzed for fatty acid composition. The one-step hexane solvent and methylation procedure[13] was used to process the samples for subsequent analysis by gas chromatography.

Botanical composition, leaf to total DM analysis and forage mass. To estimate botanical composition, leaf to total DM and forage mass available to the hens, on days 2, 6 and 10 of each treatment period, two parallel quadrats (40 × 9 cm) of forage were harvested from four random locations in each paddock. Samples were divided into three subsamples. One subsample was divided into the primary live photosynthetic tissue (leaves) and all other plant tissues. Both of the live plant tissue fractions were dried separately in an oven at 65°C to a constant dry weight and weighed. Leaf-to-total DM ratio was determined by dividing the weight of leaf DM by the weight of the total above-ground plant DM (leaf tissue + other plant tissues).

A second subsample was divided into botanical components: alfalfa, red clover, white clover, grass species, other broadleaf species and dead material. The separated materials were dried at 65°C in a forced-air oven and weighed, and used to estimate botanical composition. The remaining sample was divided into live and dead material, dried at 65°C in a forced-air oven and weighed. To estimate forage mass, the live dry weight of the three subsamples was added together [(1) leaf and other plant tissue separates; (2) botanical separates; and (3) the remaining live material] and divided by the total area sampled.

Hens and egg

Hens were weighed at the beginning and end of each 2-week period. Eggs were gathered from each treatment group daily and recorded. Hen-day egg production was calculated as: (total eggs per 14-day period/hen number × 14 days) × 100. On the last 3 days of each 2-week treatment period (days 12, 13 and 14), 11 eggs from each of the four treatment groups were chosen at random from the approximately 18–25 eggs. Each egg was weighed; yolk, egg white and shell were separated and weighed.

Since the egg fat-soluble vitamins and other lipids are entirely in the yolk, all measures of egg nutrients herein are derived from yolk analysis. Three eggs were randomly selected from the 11 eggs collected from each treatment group on each of the last 3 days of each experimental period, and the egg yolks were pooled. A total of three pooled-yolk samples per treatment (9 yolks total) were sent to the University of Missouri Experiment Station Chemical Laboratories (Columbia, Missouri, USA) for analysis of total fat, omega-3 fatty acid content and cholesterol. Egg

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yolks of the caged hens were only analyzed from periods one and three. Fat was analyzed by acid hydrolysis according to the AOAC method 954.02[14]. To quantify the fatty acids, samples were prepared by saponification–extraction in ethanolic KOH, converted to methyl esters using the boron triflouride method, and were analyzed using gas chromatography, according to the AOAC method 996.06[15]. Cholesterol was also saponified using KOH and quantified according to the AOAC method 994.10[14]. Omega-3 and omega-6 yolk weight per egg were calculated for each of the last 3 days of the experiment by multiplying the percentage of omega-3 and omega-6 fat by the percentage of total fat per egg, multiplied by the mean egg yolk weight.

Egg vitamins A and E. Five eggs were randomly selected from the 11 eggs collected from each treatment group on each of the last 3 days of each experimental period, and the five egg yolks were pooled. Three pooled yolk samples (15 yolks total) from each treatment were sent to Warren Analytical Laboratory (Greeley, Colorado, USA) for analysis of vitamins A and E (α-tocopherol). To quantify vitamin E, samples were saponified with alcoholic KOH, extracted with an organic solvent, and separated using a high pressure liquid chromatography (HPLC) silica column and florescence detection using a modification of the methods of Cort et al.[15] and Speek et al.[16,17]. For vitamin A, a 5 g yolk sample was combined with denatured alcohol (40 ml), pyrogallol solution (10 ml) and KOH (10 ml). The mixture was heated on a mantle and allowed to reflux on a condenser apparatus for 45 min. After cooling, the mixture was transferred to a 100 ml volumetric flask and was made up to 100 ml total volume with alcohol. After mixing thoroughly, a 30 ml aliquot was transferred to a clean centrifuge tube and was centrifuged for 15 min at $1200 \times \boldsymbol{g}$. An aliquot (15 ml) of the supernatant liquid was transferred to a clean 50 ml centrifuge tube and was combined with hexane (15 ml) and deionized water (10 ml). After vigorous shaking, the sample was centrifuged again as above. Two 5 ml aliquots of the hexane (top) layer were transferred to new glass culture tubes (16×100 mm) for retinol analysis. Each sample was evaporated to dryness with an N-Evaporator, and chloroform (100 µl) was added to each dry tube with mixing; then 1 ml of methanol was added and mixed. Samples were filtered through 0.45 µm discs and collected in amber glass vials. HPLC was performed with a Waters 515 pump system, 717 Autosampler, Model 470 fluorescence detector and a Nova-Pak C-18 column. Analysis was conducted with a flow-rate of 1.0 ml min^{-1}, with a column temperature of 35°C, and peak detection at 310 nm excitation and 470 nm emission. The mobile phase was 90 : 10 methanol/water and the injection volume of the sample was 50 µl (Warren Analytical Laboratory). Amounts of vitamin A and E per egg were calculated for each of the last 3 days of the experiment by multiplying the concentration of vitamins A and E by the mean egg yolk weight.

Statistical analysis

Analysis of variance was conducted on the forage data collected from each new paddock (botanical composition, forage mass, leaf to total ratio and plant fatty acids) with treatment period, pasture treatment and the interaction of treatment period by treatment as fixed effects using the GLM procedure of SAS[18]. Tukey's test was used to compare means, and means were considered different when $P < 0.05$.

Analysis of variance was also conducted on the hen and egg data (hen weight, hen-day egg production, egg and yolk weight, and egg fat composition, vitamins A and E) using PROC GLM of SAS. The experimental unit for the egg and yolk weight was the average of the eggs randomly selected from the eggs laid by the 25 hens on the last 3 days of each experimental period (days 12, 13 and 14). For the egg vitamin and fat analyses, the experimental unit was the average of the five or three pooled eggs, respectively, collected on the last 3 days of each experimental period. Since we did not collect the eggs for the analyses from the same hens, we did not conduct repeated measures analyses.

We compared the pasture treatments alone with experimental period and feed treatment as fixed effects, and group of hens that rotated among the pastures as a random effect. Tukey's test was used to compare means of the variables and differences were considered significant when $P < 0.05$. To compare the three pastured feeding treatments to the caged feed treatment, we conducted analysis of variance with PROC GLM of SAS with period and feed treatment as fixed effects. We used a pre-planned contrast to compare the pasture treatments to the caged treatment; the contrast was considered significant when $P < 0.05$. The hen-day egg production percentage data was transformed (arc sine); however, the percentage hen-day egg production data are reported herein for ease of interpretation.

Results

The average maximum and minimum temperatures, total precipitation and the 30-year normal temperatures and precipitation during the three periods of the experiment (1 July–11 August 2002) are shown in Table 2. Temperatures in 2002 during the experiment were similar to the 30-year normal temperatures. Average maximum and minimum temperatures were slightly higher than the normal temperatures in the second and third periods (largest differences were 1.7°C. between maximum temperatures and 2.5°C between minimum temperatures). Rainfall during the experiment was limited, particularly in periods 1 and 3, in comparison to the 30-year normal. Rainfall averaged eightfold lower than the 30-year normal precipitation in periods 1 and 3, and was about half the 30-year normal in period 2.

Botanical composition

The DM contribution of legume and grass did not differ ($P < 0.05$) between the alfalfa and clover legume treatments

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Table 2. Average maximum and minimum temperatures and total rainfall for each 2-week experimental period in 2002 and 30-year averages.

	Experimental period average		30-year average			
Period: dates 2002	**T-max (°C)**	**T-min (°C)**	**T-max (°C)**	**T-min (°C)**	**Experimental rainfall (cm)**	**30-year mean rainfall (cm)**
1: 7/1–7/14	28.7	16.5	27.8	16.1	0.5	4.2
2: 7/4–7/28	28.6	19.0	27.8	16.1	2.0	4.2
3: 7/28–8/11	30.3	17.7	27.7	15.9	0.4	3.8

Table 3. Botanical composition of legume, grass, other broadleaf species and dead plant tissue in the pasture treatments.

Treatment	Legume	Grass	Other broadleaves	Dead
	----------(g kg^{-1} DM)----------			
Alfalfa	730a	140b	30a	100b
Clover	700a	110b	30a	160b
Grass	0b	620a	20a	360a
P>F	0.0023	0.0063	NS	0.0001

a, b, c, designate treatments that differ significantly ($P < 0.05$) among treatments, comparisons are within columns only.
NS indicates values did not differ significantly among treatments.

(Table 3). However, the legume proportion decreased ($P = 0.002$) over the experiment, from 820 and 780 g kg^{-1} DM in periods one and two, respectively, to 530 g kg^{-1} DM in period three. In the mixed grass pastures, grasses accounted for 620 g kg^{-1} DM of the pasture DM (Table 3). Orchardgrass comprised 150 g kg^{-1} DM, smooth bromegrass 250 g kg^{-1} DM, Kentucky bluegrass 90 g kg^{-1} DM, quackgrass 80 g kg^{-1} DM, timothy 40 g kg^{-1} DM and tall fescue 10 g kg^{-1} DM of the pasture DM. Orchardgrass, smooth bromegrass and tall fescue were also the grass species present in the legume treatments. The DM contribution of the other broadleaf species did not differ ($P < 0.05$) among the three pasture mixtures; and contribution of DM from dead material was twofold higher ($P = 0.0001$) in the grass treatment than in the legume treatments (360 versus 110 and 160 g kg^{-1} DM; Table 3). For most of the experiment, the three treatments were in vegetative stages of development. The grass treatment was vegetative throughout the experiment; the alfalfa was mid-vegetative, without flowers or buds. Red and white clovers were also in the mid-vegetative stage in periods one and three, but in the second period many plants were in the early flowering stage.

Forage mass, leaf to total DM ratio and plant fatty acids

The interaction of treatment and period was significant for forage mass ($P = 0.02$, Fig. 1A). In the first period, the legume treatments averaged 3182 kg ha^{-1}, three times more forage mass than in the grass treatment. In the second period, the forage mass did not differ between the alfalfa and grass treatments, but the clover treatment had twice as much forage mass as the grass treatment. Forage mass did not differ among the two legume treatments in any period, or among all treatments in the last period.

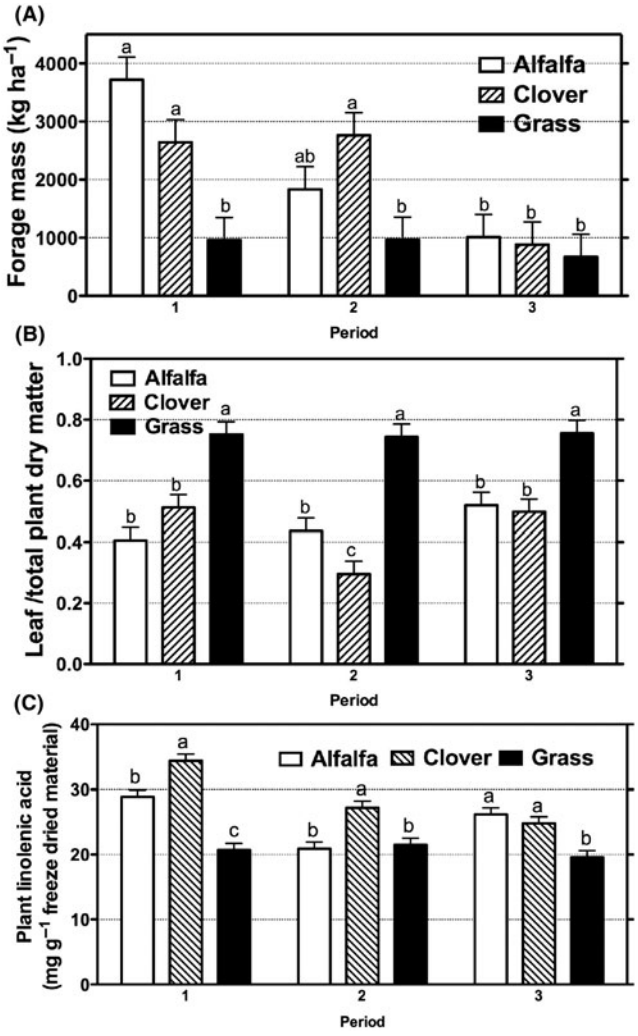

Figure 1. Forage characteristics of the three pasture plant treatments during the three 2-week experimental periods, with standard error bars. (A) Forage mass, (B) leaf to total aboveground DM ratio, and (C) forage linolenic acid concentration. a, b, c designate treatments that differ significantly ($P < 0.05$) within periods.

The interaction of treatment and period was also significant for the leaf to total DM ratio ($P < 0.05$,

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Table 4. Mean egg and yolk weight, amount of omega-6 and omega-3 fatty acids, and cholesterol, vitamins A and E per egg yolk; and concentration of omega fatty acids cholesterol, vitamins A and E concentration per egg yolk in the three pasture treatments.

Pasture feed	Alfalfa	StdErr	Clover	StdErr	Grass	StdErr
			(g)			
Egg weight	54.71	0.806	54.48	0.806	52.34	0.806
Yolk weight	11.14	0.183	11.64	0.183	10.93	0.183
Omega-6 fats/egg yolk	0.55	0.019	0.60	0.019	0.56	0.019
Omega-3 fats/egg yolk	0.12	0.009	0.13	0.009	0.10	0.009
Cholesterol/egg yolk	16.5	0.54	17.8	0.54	16.9	0.54
			(IU)			
Vitamin A/egg yolk	330	27	340	27	300	27
Vitamin E/egg yolk	1.2ab	0.11	1.1b	0.11	1.3a	0.11
			(g per 100 g egg yolk)			
Linolenic (ωC18:3)	1.70	0.111	1.61	0.111	1.22	0.111
Stearidonic (ω18:4)	0.09	0.019	0.09	0.019	0.04	0.019
EPA (ω20:5)	0.04	0.003	0.04	0.003	0.04	0.003
C22:1	0.027a	0.0023	0.032a	0.0023	0.003b	0.0023
DPA (ωC22:5)	0.27	0.027	0.22	0.027	0.23	0.027
DHA (ω22:6)	1.69	0.102	1.63	0.102	1.50	0.102
Total omega-3 fats	3.76	0.213	3.58	0.213	3.03	0.213
Total omega-6 fats	16.64	0.147	16.98	0.147	16.90	0.147
Cholesterol	1.48	0.028	1.51	0.028	1.54	0.028
			(IU per 100 g egg yolk)			
Vitamin A	2900	187	2900	187	2700	187
Vitamin E	10.8ab	0.21	9.6b	0.21b	11.8a	0.20

Omega-3 fatty acids are bold. a, b, c designate treatments that differ significantly ($P < 0.05$) among pasture treatments.
DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid.

Fig. 1B). In the grass pastures, leaf to total DM ratio averaged 0.75 and was higher than the legumes in all periods. Leaf to total DM ratio for the legume treatments was similar in the first and third periods (averaging 0.45 and 0.50, respectively), but in the second period, the ratio dropped to 0.30 in the clover treatment, while remaining similar in the alfalfa treatment. This decrease probably occurred because the clover plants had begun flowering in second period, while the alfalfa treatments remained vegetative.

There was also a significant interaction of treatment and period for the concentration of linolenic acid (C18:3) in the plant tissues ($P = 0.0001$, Fig. 1C). In period one, clover had 20% more C18:3 than alfalfa and 70% more than the grass treatment. Linolenic acid concentrations were similar in alfalfa and grass in period two, and were 24% lower than in the clover treatment. In period three, the concentration of C18:3 decreased in the clover treatment to a level similar to the alfalfa pasture; but the legume treatments still had 30% more C18:3 than the grass treatment.

Hens

Livability at the end of the study was 100% for the pastured hens and 97.9% for the caged hens ($P < 0.0001$). Body weight of the pastured birds, while not significantly different at the beginning of the study (1540 g at 19 weeks), was influenced by the period of lay and treatments. At the end of the study the weight of the pastured hens did not differ significantly, but the caged hens weighed significantly ($P < 0.0001$) more than the pastured birds (1821 versus 1571 g) and closer to the 24-week of age breed-age standard[19]. Hen-day egg production did not differ among pasture treatments, but differed ($P = 0.0035$) between the caged hens and the pasture hen treatments, and over the three experimental periods ($P < 0.05$). Caged hens averaged 85% egg production over the three 2-week periods, and pastured birds averaged a significantly lower 72%. In both treatments, hen-day egg production was lowest in the first period (56%), and increased in the second (80%) and third periods (90%).

Egg mass and yolk fat composition

Egg weight, yolk weight, and the concentration of fat, cholesterol and total omega-6 fatty acids did not differ among the pasture treatments ($P < 0.05$, Table 4), nor among the pastured and caged treatments (data not shown), although they increased from the first period to the third period (Table 5). The concentration of the omega-3 fatty acids and the total amount of omega-3 did not differ, either among the pasture treatments (Table 4) or among the experimental periods (Table 5). Erucic acid (22:1) was the only fatty acid that differed in concentration among the eggs of hens that foraged on the pastured treatments, with lower concentrations in the eggs of the hens that foraged on grass (Table 4).

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Table 5. Mean egg and yolk weight, amount of omega-6 and omega-3 fatty acids and cholesterol per egg yolk; and concentration of vitamins A, E and cholesterol in the egg yolks of all feeding treatments in the three experimental 2-week periods.

	1		2		3	
Period	**Mean**	**StdErr**	**Mean**	**StdErr**	**Mean**	**StdErr**
	(g per egg)					
Egg weight	50.89b	0.656	54.60a	0.803	56.15a	0.656
Yolk weight	10.37b	0.250	11.62ab	0.306	12.20a	0.250
Omega-6 fats	0.51b	0.023	0.58ab	0.028	0.64a	0.023
Omega-3 fats	0.10	0.005	0.09	0.006	0.11	0.005
Cholesterol	15.4b	0.32	17.2a	0.40	18.6a	0.32
	(IU per egg)					
Vitamin A	252b	21.0	300a	25.7	353a	21.0
Vitamin E	0.8b	0.07	1.0ab	0.09	1.0a	0.07
	(g per 100 g egg yolk)					
Cholesterol conc.	1.50	0.03	1.49	0.03	1.50	0.03
	(IU per 100 g egg yolk)					
Vitamin A conc.	2400	180	2600	220	2900	180
Vitamin E conc.	8	0.6	10	0.8	10	0.6

a, b, c designate treatments that differ significantly ($P < 0.05$) among periods.

Table 6. Fatty acid profile of the eggs yolks from hens on alfalfa, clover, and grass pastures and caged hens, averaged across the three pasture experimental periods.

	Pastured				**Pastured versus caged contrast significance**
	Alfalfa	**Clover**	**Grass**	**Caged**	
	($g\ kg^{-1}$ yolk)				
Total fat	298	304	300	305	NS
	(% of total yolk fatty acids)				
Mryistic (14:0)	0.33	0.32	0.33	0.31	NS
Myristoleic (14:1)	0.030	0.027	0.030	0.029	NS
Palmitic (16:0)	24.86	24.16	24.58	24.26	NS
Palmitoleic (16:1c)	3.50	3.37	3.38	3.11	NS
Stearic (18:0)	7.86	7.67	7.67	8.02	NS
Elaidic (18:1t)	0.52	0.51	0.48	0.63	0.039
Oleic (18:1c)	38.73	38.92	38.86	41.99	0.0015
Linoleic (C18:2)	14.54	15.02	14.84	14.21	0.0339
Linolenic (ω18:3)	1.67	1.61	1.22	0.33	0.0012
Stearidonic (ω18:4)	0.09	0.09	0.04	0.07	NS
Arachidic (20:0)	0.04	0.04	0.04	0.05	NS
Arachidonic (20:4)	1.97	1.96	2.06	2.10	NS
EPA (ω20:5)	0.04	0.04	0.04	0.02	0.0022
Docosanoic (22:0)	0.127	0.117	0.128	0.137	0.0035
Erucic (22:1)	0.03	0.03	0.00	0.00	NS
DPA (ω22:5)	0.27	0.22	0.23	0.10	0.005
DHA (ω22:6)	1.69	1.63	1.50	0.76	0.0014
Ligonceric (24:0)	0.04	0.03	0.04	0.04	NS
Total omega-3	3.76	3.59	3.03	1.28	0.0008
Total omega-6	16.50	16.98	16.90	16.31	NS
Omega-6/omega-3	4.44	4.76	5.70	12.05	< 0.0001

Omega-6 fatty acids are italicized and omega-3 fatty acids were bold.
NS indicates pre-planned contrasts that were not significant.
DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid.

The concentrations of the omega-3 fatty acids, however, were higher in the eggs of the pastured hens than the caged hens, with 4.5-fold more linolenic acid ($P = 0.0012$), twofold more eicosapentaenoic acid (EPA) ($P = 0.0022$) and docosahexaenoic acid (DHA) ($P = 0.0014$) and 2.4-fold more docosapentaenoic acid (DPA) ($P = 0.0050$) than the

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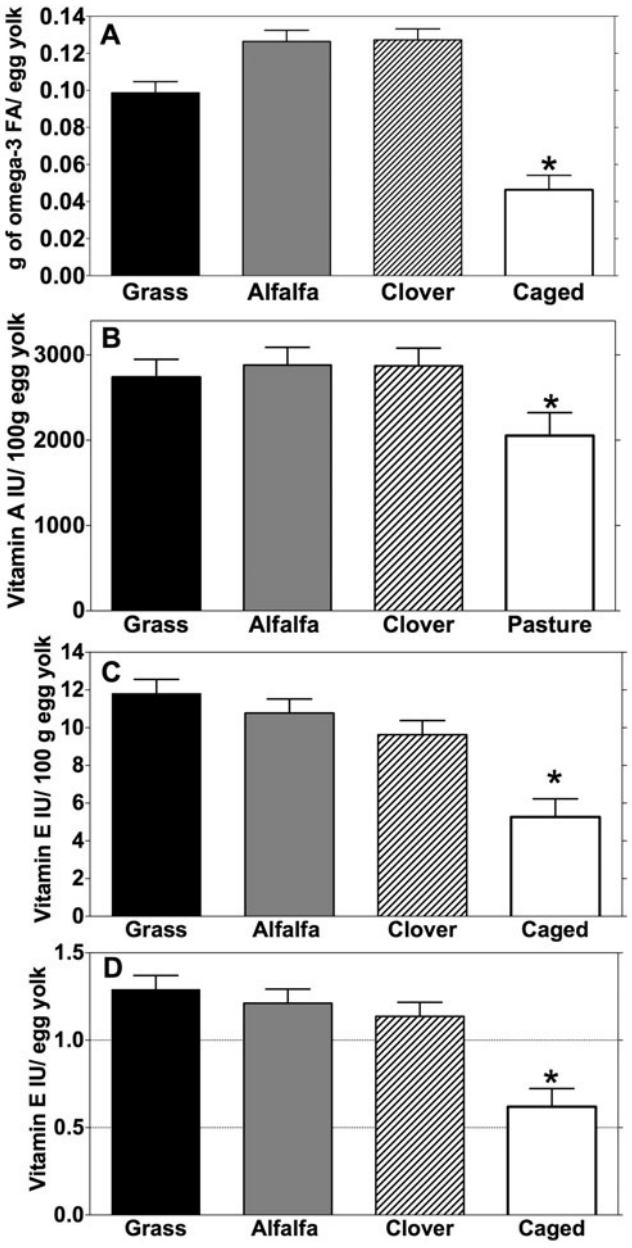

Figure 2. Egg yolk characteristics of the four feeding treatments averaged over the three 2-week experimental periods with standard error bars: (A) omega-3 fatty acid amount (mg) per egg yolk, (B) vitamin A concentration of the egg yolks, (C) vitamin E concentration in the egg yolks and (D) vitamin E amount of the egg yolks. *Indicates that the pre-planned contrast of the pastured treatments versus the caged treatments was significant ($P < 0.05$).

caged hens' eggs (Table 6). In total, the pastured hens' eggs had 2.7-fold higher concentration of omega-3 fatty acids ($P = 0.0008$) than the caged hens', and the ratio of omega-6 to omega-3 fatty acids was 2.4-fold lower ($P < 0.0001$) in the pastured eggs than in the caged hens' eggs. In addition, the total amount of omega-3 fat (g per egg yolk) of the pastured hens was 2.5-fold higher than that of the caged hens ($P = 0.0007$; Fig. 2A). In addition, compared to the eggs of the caged hens, the eggs of the pastured hens had a 4% higher ($P = 0.0339$, Table 6) concentration of the omega-6, linoleic acid (C18:2), and lower concentrations ($P < 0.05$, Table 6) of elaidic (18:1t), oleic (18:1c) and docosanoic (22:0) acids.

Egg yolk vitamins

Although egg yolk vitamin A concentration and total amount per egg did not differ among hens that foraged on the pasture treatments, the eggs of the hens that foraged on pasture averaged a 38% higher concentration of vitamin A (IU per 100 g egg yolk) than the eggs of caged hens (Fig. 2B). The total amount of vitamin A (IU per egg yolk), however, did not differ among feed treatments (data not shown) but was significantly higher in period 3 of the experiment than in first period ($P < 0.05$, Table 5).

When only the pasture treatments were compared, the concentration (IU per 100 g egg yolk) and total amount of vitamin E (IU per egg yolk) in the eggs of the hens that foraged on grass were 14 and 23% higher, respectively, than in the eggs of the hens that foraged on clover ($P < 0.05$, Table 4). Further, eggs of pastured hens had twice the concentration and total amount of vitamin E per egg of the caged hens (Fig. 2C and D, respectively). Vitamin E concentration (IU per 100 g egg yolk) did not differ among periods, but the amount of vitamin E per egg (IU per egg yolk) was significantly higher in the second and third periods of the experiment than in the first period (Table 5, $P < 0.05$).

Discussion

There was some variation in forage mass among the pasture treatments and experimental periods, with the grass treatment often producing a higher leaf to total plant ratio. None of these forage characteristics, however, had a pattern that explained the variation in plant linolenic acid concentrations (Fig. 1). Forage samples of the pasture treatments differed in linolenic acid concentration (C18:3), although eggs from hens foraging the pasture treatments did not differ in total linolenic acid or total omega-3 fatty acid composition. This suggests that the hens selectively foraged to obtain similar fatty acid diets from all of the pasture treatments, or that the experimental forage and plant linolenic acid concentration differences were not large enough to influence hen egg omega-3 fatty acid composition.

The only differences observed among eggs of hens that foraged the pasture treatments were in erucic acid (22:1) and vitamin E (Table 4). Eggs of hens that foraged on grass produced eggs with less erucic acid and a 14% higher concentration of vitamin E and 23% more vitamin E in total per egg, suggesting that grasses may provide hens with more vitamin E than clover. Richardson et al.[20] also found that grass silage enhanced beef vitamin E concentration compared to red clover silage, or a red clover and grass silage mix (50:50 mix) when fed to beef cattle. The meat of the cattle fed grass silage had significantly more vitamin E

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than the cattle fed red clover or the red clover and grass silage mixture.

The eggs of the caged hens and the pastured hens did not differ in weight, total fat, cholesterol and omega-6 fatty acids. The eggs of the pastured hens, however, had significantly higher concentrations of vitamins A and E and omega-3 fatty acids. Vitamin A concentrations were almost 40% higher in the pastured hens than the caged hens' eggs, but the total amount of vitamin A per egg did not differ between the pastured and caged hens. Although egg and yolk weights did not differ statistically among the treatment hens, the lack of difference in vitamin A per egg suggests that egg weight variation among feed treatments and the observed increase in egg weight over the experimental periods countered the vitamin A concentration differences. On the other hand, the small sample size may also have limited the power of the statistical tests.

The pastured hens' eggs also had almost twice as much total vitamin E (IU per egg yolk) and total omega-3 fatty acids per egg; and the ratio of omega-6 to omega-3 fatty acid was less than half the ratio of the caged hens' eggs. Concentrations of vitamin E (IU per 100 g egg yolk) and long-chain omega-3 fatty acids (EPA, DPA and DHA) were twice as high in the pastured hens' eggs, and the total omega-3 fatty acid concentrations were 2.7-fold higher than in the caged hens' eggs. These results are similar to the findings of Tolan et al.[9], in the UK, and particularly to those of Lopez-Bote et al.[10], in Spain, who found that hens given access to grassland produced eggs that had almost three fold more omega-3 fat and 30% more vitamin E than the eggs of caged hens. Further, the omega-3 fatty acid and vitamin E egg concentrations that Lopez-Bote et al.[10] measured in Spain, were in a range similar to the values measured herein. The omega-6 to omega-3 fatty acid ratio in the caged and free-range hens (5.21 versus 18.73, free-range versus caged[7]) was also similar to the ratios in this study (5.0 versus 12.05, pastured versus caged).

The caged hens consumed an average of 113 g mash $hen^{-1}\,day^{-1}$, typical for Hy-Line brown egg hens at this age[19]. With the daily allotment of 70 g mash $hen^{-1}\,day^{-1}$ (11.8 g CP, and 200 kcal ME) to pastured hens, hen-day egg production was 15% lower than the caged hens and their body weight at the end of the experiment was 14% lower than the caged hens. Hens did not forage to the degree necessary to meet their requirements for energy and protein when compared to the caged birds. Although egg production and body weight were greater than that expected of birds maintained on only 70 g mash $hen^{-1}\,day^{-1}$, they only derived approximately 13.2% of their energy requirement (36 kcal ME) and 21.5% of their CP needs (3.9 g) from pasture foraging. The pastured hens were still lacking dietary protein and energy (approximately 2.3 g CP and 35 kcal ME) on a daily basis to match the intake of the caged hens. Buchanan et al.[21] found that Leghorn roosters only obtain small amounts of energy from pasture forage (285–463 kcal kg^{-1} forage, as is basis). And previous work with broiler chickens has shown they are unable to overcome a 7% reduction in dietary energy with supplemental forage[22]. We have since estimated that, at the level of voluntary forage consumption of hens in this study, pastured hens would require more than 13 g additional mash feed to sustain body weight and egg production equal to that of the caged hens. Although 83 g $hen^{-1}\,day^{-1}$ (70 g + 13 g) would most likely provide the CP needs for the pastured birds to maintain similar egg production and body weight to the caged birds, additional mash would be required to provide needed energy to support the greater activity of pastured hens. Supplementing the birds with additional mash, however, would likely result in reduced omega-3 fatty acid and vitamin A and E concentrations in their eggs. Supplementing with chia (*Salvia hispanica* L.), flaxseed (*Linum usitatissimum* L.)[23], fish meal, or another feed ingredient high in omega-3 fatty acids would be another means to increase the total omega-3 fat concentration and decrease the omega-3 to omega-6 ratio in the hens' diet and eggs. Further research is needed to identify how to optimize pastured poultry feed supplementation for optimum egg production, hen welfare and egg nutritional quality.

In summary, although the pasture mixtures differed in linolenic acid concentration, the eggs from hens moved among the pasture treatments did not differ in linolenic acid or total omega-3 fatty acid concentration. In comparison to eggs of caged birds, the pastured hens' eggs had twice as much vitamin E and long-chain omega-3 fatty acids, and 2.5-fold more total omega-3 fatty acids. Pastured hens, however, also weighed less and produced fewer eggs than caged hens, indicating that they should be supplemented with more dietary energy and crude protein, which could reduce the observed egg omega-3 fat and vitamins A and E concentrations.

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