

Omega-3 enriched eggs: The influence of dietary α -linolenic fatty acid source on egg production and composition¹

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Ayerza, R. (h) and Coates, W. 2001. **Omega-3 enriched eggs: The influence of dietary α -linolenic fatty acid source on egg production and composition.** Can. J. Anim. Sci. 81: 355–362. A study was conducted to assess the effect of replacing chia with flaxseed as a source of α -linolenic acid in laying hen feed. Five diets, identified as T0 through T4, containing 0-0, 7-3, 9-5, 11.5-2.5 and 14-0% whole chia seed and whole flaxseed, respectively, were fed to 240 White Shaver laying hens, at 60 wk of age. No difference ($P > 0.05$) in egg production, egg weight, yolk weight and albumen weight were found among treatments. Total ω -3 acid percentage was higher ($P < 0.05$) in the yolks from the hens fed the α -linolenic acid-enriched diets, than in those fed the control diet. Of the three treatments that had any combination of chia and flaxseed comprising 14% of the diet, T2 yielded a lower ($P < 0.05$) ω -3 content in the yolk, than did T3 and T4. A taste panel found no difference ($P > 0.05$) in flavor or off-flavor among treatments; however, panel preferences were lower for eggs produced by hens fed the highest level of flaxseed (T2). This study showed no advantages to replacing chia with flaxseed to produce ω -3 enriched eggs. Greater availability of flaxseed, however, might make it more attractive in some markets.

Key words: Eggs, chia, flaxseed, omega-3 fatty acid

Ayerza, R. (h) et Coates, W. 2001. **Oeufs enrichés avec omega 3: l'influence de la source des acides gras alpha-linolenic dans une diète, pour la production et composition des oeufs.** Can. J. Anim. Sci. 81: 355–362. On a réalisé une étude pour déterminer l'effet produit, en remplaçant le chia par le sésame de ligne comme une source d'acide alpha-linolénique dans la nourriture des poules pondeuses. On a nourri 240 poules pondeuses White Shaver âgées de 60 semaines, avec cinq diètes, identifiées comme T0 à T4, qui contiennent 0-0, 7-3, 9-5, 11.5-2.5 et 14-0 % combinaisons de chia totale et du sésame de ligne totale, respectivement. On n'a pas trouvé aucune différence ($P > 0.05$) dans la production de l'oeuf, poids de l'oeuf, poids du jaune d'oeuf, et poids de l'albumen, parmi les traitements. Le pourcentage de l'acide oméga-3 a été plus haut ($P < 0.05$) dans les jaunes d'oeuf des poules nourries avec les diètes enrichies avec de l'acide alpha-linolénique que celles nourries avec la diète contrôle. En faisant la comparaison parmi les traitements qui ont quelque combinaison du chia et du sésame de ligne, T2, 14 % de la diète, a eu moins contenu d'oméga-3 ($P < 0.05$) dans le jaune d'oeuf que les traitements T3 et T4. Un panneau d'essai n'a pas trouvé aucune différence ($P > 0.05$) dans le saveur ou le goût anormal parmi les traitements; cependant, des préférences du panneau ont été moins, pour les oeufs produits par les poules nourries avec un niveau majeur du sésame de ligne (T2). Cet étude n'a pas démontré l'avantage de remplacer le chia avec le sésame de ligne pour produire oeufs enrichis avec d'oméga-3. Cependant, la plus grande disponibilité du sésame de ligne, peut le faire plus attractive dans quelques marchés.

Mots clés: Oeuf, chia, line, omega-3, acide gras

A number of epidemiological and controlled experiments have reported an inverse relationship between ω -3 acid consumption, and risk of cardiovascular disease (Bang et al. 1980; Kromhout 1985; Kinsella et al. 1990; Pauletto et al. 1996; Lorgeril et al. 1996; Leaf and Kang 1998). Western diets are deficient in ω -3 fatty acids and high in ω -6 fatty acids. The main reason for this imbalance is consumers' increasing consumption of vegetable oils such as sunflower, corn and safflower, which are rich in ω -6 poly unsaturated fatty acids (PUFAs) (Simopoulos and Robinson 1998).

The fatty acid composition of eggs can be changed by modifying the fatty acid composition of the hens' diet. Such changes have been demonstrated in a number of studies (Caston and Leeson 1990; Cherian and Sim 1991; Caston et al. 1994; Farrell 1994; Cherian et al. 1995; Nash et al. 1995; Scheideler et al. 1998b; Ayerza and Coates 1999).

Currently marketed ω -3 enriched eggs are produced by including flaxseed, chia seed, fish oil or meal, or marine algae in the hens' diet. Both seeds are rich in α -linolenic acid, while the marine sources contain the long chain ω -3 acids, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA).

A number of researchers have reported a fish smell or taste with eggs produced by hens fed marine products or flaxseed (Adam et al. 1989; Lee et al. 1991; Jiang et al. 1992; Van Elswyk et al. 1992; Caston et al. 1994; Nash et al. 1995; Van Elswyk et al. 1995; Scheideler et al. 1997). The flavor is thought to arise through oxidation of polyunsatu-

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rated fatty acids (Jiang et al. 1992; Van Elswyk et al. 1995; Cherian et al. 1996). Other researchers, however, have suggested that the fishy odor arises from both lipid and non-lipid substances present in the feed (Leskanich and Noble 1997).

Of the components used in hen diets to produce ω -3 rich eggs, only flax (*Linum usitatissimum* L.) and chia (*Salvia hispanica* L.) are produced via traditional agricultural practices. These two species have the highest concentration of ω -3 α -linolenic acid (Ayerza 1995; Oomah and Kenasehuk 1995; Coates and Ayerza 1996, 1998). There are, however, marked differences in availability and price between these two α -linolenic sources. Flaxseed is a commodity and hence is easy to find in world markets. Chia, however, is an emerging crop and its availability does not meet current demand.

Owing to the availability of flax as well as its relatively low price, there have been many attempts to use it as an ω -3 fatty acid source in poultry rations. Several publications have shown the negative effects that antinutritional components of flax have had on the growth of layers and broilers and on egg production. Caston et al. (1994) found that as flax content in the diet increased, hen body weight decreased. They showed that hens fed a 20% flax diet were significantly lighter than those fed a 10% diet, which in turn were lighter than hens fed the control diet. Scheideler and Froning (1996) found egg weight to respond to dietary treatments, with a significant ($P < 0.01$) decrease in egg size reported for the 5 and 15% flaxseed diets relative to the control and fish oil diets. Ayamond and Van Elswyk (1995) reported that egg production from hens fed flaxseed generally decreased over time, with a 15% flaxseed diet showing decreased production as early as 2 wk into the test. These negative effects have been attributed to antinutritional components in flaxseed (Kung and Kummerow 1950; Homer and Schaible 1980; Bell 1989; Bhatti 1993; Bond et al. 1997).

None of the toxic factors found in flax have been found in either chia seed or chia seed oil (Bushway et al. 1984; Ting et al. 1990; Lin et al. 1994; Ayerza and Coates 1999). Hence chia has a definite advantage over flax as a poultry feed.

The objectives of this study were to determine the effect replacing chia seed with flaxseed would have on hen performance, egg fatty acid composition, egg cholesterol and total fat content as well as on egg sensory attributes.

MATERIALS AND METHODS

Two hundred and forty White Shaver laying hens, at 60 wk of age, were selected for the study. The hens were housed three to a cage (30 \times 45 \times 43 cm tall at the front, and 38 cm tall at the back) in a commercial egg production facility in San Vicente, Province of Buenos Aires, Argentina. Production practices at the facility were adopted for the test, with the hens being randomly selected from the commercial flock. The diets contained 0-0, 7-3, 9-5, 11.5-2.5 and 14-0% combinations of whole chia seed and whole flaxseed (T0, T1, T2, T3 and T4), respectively. The diets were formulated with two factors in mind. The first was to keep flax content below 5%, to ensure that it would not affect hen performance nor impart undesirable organoleptic characteristics to

the eggs. Values greater than 5% have been found to negatively impact egg appearance and flavor (Scheideler et al. 1997; Leeson et al. 1998). The second consideration was to keep α -linolenic levels in the diets relatively uniform, without significantly affecting the nutrient balance of the rations. T1 was included to determine if a combination of comparatively low levels of both seeds might have a complementary effect different from diets containing high levels of both seeds. Nutrient composition of the diets is shown in Table 1. Each diet (treatment) had 16 replicates, with one cage of three hens comprising each replicate.

Following random allocation to the cages, the hens were fed for 30 d, prior to collecting data. The trial then lasted 30 days, with each cage receiving 345 gm of feed per day. This followed the commercial feeding practice at the facility. Water was provided for ad libitum consumption.

Eggs were collected daily, counted and weighed individually. Four eggs from each treatment were randomly selected on day 30 for analysis. The eggs were weighed and opened, and the yolks were separated, weighed and then frozen. To assess the influence of the various diets on hen performance, all of the hens from each treatment were weighed on day 0 and on day 30.

Sensory Evaluation

A taste test was conducted to evaluate preference and flavor. The test used eggs collected during the last week of the trial from hens fed each of the five diets. All of the eggs were prepared in the same manner, 2 h before the taste test began. Eggs from each treatment were placed in water at room temperature, brought to boiling, then taken from the pot and placed under cold running water for 10 min. The eggs were peeled, cut longitudinally and placed in separate containers.

Twenty untrained adult panelists (16 male and 4 female) were chosen for the test. Each panelist individually received five plates, each containing half an egg randomly selected from each of the containers. Each plate was marked with an A, B, C, D or E. These corresponded to the five diets, T0, T1, T2, T3 and T4, respectively.

Panelists sat one meter apart at a table, and received the five plates in a random order. Each panelist had a sheet of paper containing five labeled squares (one for each egg) to fill in using the evaluation criteria described by Caston et al. (1994) and Ayerza and Coates (1999). The ratings were as follows:

- **Preference:** like very much, like moderately, neither like nor dislike, dislike moderately, dislike very much
- **Flavor:** high intensity, intense, low intensity, very low intensity, normal.

For scoring and analysis purposes, each classification was assigned a number from one to five. Cold tap water was provided for panelists to rinse their mouths between samples.

Laboratory Analyses

The frozen yolks were thawed, mixed and then analyzed for cholesterol content, total fat content and fatty acid composition. Total lipids were extracted with a chloroform/methanol mixture (2:1 vol/vol) (Folch et al. 1957). Lipid content was determined gravimetrically. Total lipids were converted to

Table 1. Nutrient composition of the laying hen diets

Ingredient	Chia (%)	0	7	9	11.5	14
	Flax (%)	0	3	5	2.5	0
Treatment	T0	T1	T2	T3	T4	
ME (kcal kg ⁻¹)	2730	2780	2780	2780	2780	2780
Crude protein (%)	17.3	16	16.3	16.1	16.7	16.7
Lysine (%)	0.83	0.88	0.87	0.87	0.86	0.86
Methionine + Cystine (%)	0.65	0.7	0.7	0.7	0.66	0.66
Threonine (%)	0.63	0.66	0.66	0.66	0.65	0.65
Tryptophan (%)	0.18	0.2	0.2	0.2	0.2	0.2
Lipids (%)	4.32	7.4	8.28	8.28	7.18	7.18
Fiber (%)	4.05	5.3	5.89	6.51	7.11	7.11
Calcium (%)	3.7	3.7	3.7	3.7	3.7	3.7
Available P (%)	0.4	0.41	0.41	0.41	0.41	0.41
Ca:P ratio	5.44	5.15	5.09	5.04	4.99	4.99
Xanthophylls (ppm)	14.93	29.54	29.1	29.06	29.19	29.19
Miristic ^z	0.5	<0.1	<0.1	<0.1	<0.1	<0.1
Palmitic ^z	13.1	8.6	8.36	8.74	8.94	8.94
Palmitoleic ^z	0.16	0.24	0.2	0.22	0.21	0.21
Stearic ^z	5.5	3	3.3	2.6	2.4	2.4
Oleic ^z	30.1	18.6	17.5	15.9	16.4	16.4
Linoleic ^z	46.4	37.7	32	35	38	38
α -Linolenic ^z	3.38	29.9	36.6	33.9	31.9	31.9
Linoleic: α -linolenic ratio	13.72	1.26	0.87	1.03	1.19	1.19
Arachidonic ^z	0.17	0.2	0.19	0.16	0.16	0.16
Gadoleic ^z	0.18	0.13	0.14	0.1	0.1	0.1

^zPercentage of total fat.

fatty acid methyl esters using the IRAM 5650II method (Instituto Argentino de Racionalización de Materiales 1982) which is equivalent to ISO 5508 (1990). Fatty acid methyl esters were separated and quantified by an automated gas chromatograph (Model 6890 GC, Hewlett Packard Co, Willmington, DE) equipped with flame ionization detectors and a 30 m \times 530 μ m i.d. capillary column (Model HP-FFAP, Hewlett Packard Co, Willmington, DE). HP Chem Station (Hewlett Packard Co, Willmington, DE) was used to integrate peak areas.

Cholesterol was extracted according to the Association of Official Analytical Chemist's procedure 941.09 (1990). An automated gas chromatograph (Model 6890 GC, Hewlett Packard Co, Willmington, DE) equipped with flame ionization detectors and a Model HP-1 Methyl Siloxane capillary column (Hewlett Packard Co, Willmington, DE) was used to quantify the cholesterol levels.

Statistical Analysis

The feeding trial was set up as a randomized block design, with the experimental unit being one cage of three hens. Each variable was compared using the Generalized Linear Model analysis of variance technique to assess treatment differences. When the *F*-value was significant ($P < 0.05$), differences in means were analyzed for significance using Duncan's Multiple Range Test (SAS Institute, Inc. 1988).

RESULTS

Hen and Egg Data

Hen weight, weight gain, egg weight, yolk weight and albumen weight are presented in Table 2. No significant differences ($P > 0.05$) in egg production, egg weight, yolk weight

or albumen weight were found among treatments. Hen weight appeared to be affected by the level of flax in the diet. However, a significant ($P < 0.05$) difference was detected only between T2 and T3. Only treatment T2, the diet containing the highest flaxseed content, resulted in a weight loss.

Cholesterol and Total Lipids

Cholesterol and total lipid content of the yolks are presented in Table 3. No significant difference ($P > 0.05$) in cholesterol was found among treatments. Lipid content of the yolks was lowest for the three highest dietary levels of α -linolenic acid. Differences in yolk lipid contents were significant ($P < 0.05$) only between T1, and T2 and T4.

Fatty Acid Composition

Fatty acid composition of the yolks, as a percentage of total fatty acid content, is presented in Table 3.

Saturated Fatty Acids

Myristic acid percentage was not significantly different ($P > 0.05$) among treatments, but palmitic and stearic percentage were significantly different ($P < 0.05$). Palmitic acid percentage was lowest for the three highest α -linolenic diets, while stearic percentage was highest with the diet containing the highest level of flaxseed. The differences in palmitic acid content were significant ($P < 0.05$) between the T1 and the T2, T3 and T4 diets. For stearic acid, the differences were significant ($P < 0.05$) between T2, and the T0, T3, and T4 diets. Total saturated fatty acid percentage, calculated as the sum of myristic, palmitic and stearic acids, was not significantly different ($P > 0.05$) among treatments (Table 4).

Table 2. Hen weight, hen weight gain, egg production, egg weight, yolk weight and albumen weight for laying hens fed different levels of chia and flaxseed

Treatment	Chia (%)	Flax (%)	Production (egg per hen per day)	Hen weight		Weight gain (g)	Egg weight (g)	Yolk weight (g)	Albumen weight (g)
				Day 0 (g)	Day 30 (g)				
T0	0	0	0.76	1651	1656 ^{ab}	4.63 ^{ab}	68.87	18.55	41.81
T1	7	3	0.74	1647	1660 ^{ab}	13.71 ^{ab}	66.65	19.01	39.25
T2	9	5	0.74	1637	1614 ^b	-23.79 ^b	68.88	18.16	42.49
T3	11.5	2.5	0.75	1644	1689 ^a	45.04 ^a	67.16	19.8	39.43
T4	14	0	0.72	1648	1670 ^{ab}	22.29 ^{ab}	68.15	18.83	41.46
cr ²			0.06	21.2	67.3	68.4	10.7	3.05	9.7

²Critical range for mean separation.^{a, b} Means within a column lacking a common letter differ ($P < 0.05$) according to Duncan's multiple range test.**Table 3. Cholesterol content, total lipids content and fatty acid composition of egg yolk**

Treatment	Chia	Flax	Lipids ² (%)	Cholesterol (g per 100 g yolk)	Fatty acids (% of total fatty acids)							Arachidonic	DHA ³
					Myristic	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic	Linolenic		
T0	0	0	10.75 ^{ab}	1.3	0.33	25.15 ^{ab}	4.18 ^a	8.38 ^b	35.88 ^a	18.70 ^b	0.75 ^d	2.14 ^a	1.10 ^b
T1	7	3	11.25 ^a	1.29	0.35	25.83 ^a	3.27 ^b	8.92 ^{ab}	31.88 ^b	19.48 ^b	6.95 ^c	1.03 ^c	1.07 ^b
T2	9	5	9.26 ^b	1.16	0.28	22.78 ^b	2.80 ^b	9.67 ^a	27.85 ^c	21.80 ^a	9.16 ^b	1.38 ^b	1.77 ^a
T3	11.5	2.5	9.79 ^{ab}	1.19	0.3	22.73 ^b	2.84 ^b	8.44 ^b	27.13 ^c	22.43 ^a	11.73 ^a	0.82 ^c	1.13 ^b
T4	14	0	9.59 ^b	1.15	0.35	24.08 ^b	2.81 ^b	8.65 ^b	27.25 ^c	21.38 ^a	11.65 ^a	0.73 ^c	1.02 ^b
cr ²			1.64	0.17	0.08	2.63	0.53	1.06	2.46	1.65	0.97	0.34	0.43

²Percentage based on total egg weight.³Critical range for mean separation.⁴Docosahexaenoic acid.^{a-d} Means within a column lacking a common letter differ ($P < 0.05$) according to Duncan's multiple range test.

Monounsaturated Fatty Acids

Palmitoleic and oleic acids were significantly lower ($P < 0.05$) in yolks from the hens fed the α -linolenic diets than in yolks from hens fed the control diet. Oleic acid content was significantly higher ($P < 0.05$) for hens fed the T1 diet than for the hens fed the other α -linolenic enriched diets.

Total monounsaturated fatty acid percentage, calculated as the sum of palmitoleic and oleic acid percentage, was significantly lower ($P < 0.05$) in the yolks from hens fed the α -linolenic enriched diets than in the yolks from hens fed the control diet. Also, the three highest α -linolenic acid treatments, T2, T3 and T4, gave a significantly lower ($P < 0.05$) total monounsaturated fatty acid content than did the T1 diet (Table 4).

Polyunsaturated Fatty Acids

Addition of α -linolenic acid to the diet increased the percentage of total polyunsaturated fatty acids in yolks relative to the control diet ($P < 0.05$; Table 4). Polyunsaturated fatty acid contents of the three highest α -linolenic acid diets were not significantly different ($P > 0.05$); however, they were significantly higher than T1 ($P < 0.05$).

The percentage of arachidonic acid, an ω -6 PUFA, was significantly lower ($P < 0.05$) in the yolks produced by hens fed the α -linolenic enriched diets than in the yolks of hens fed the control diet (Table 3). As flaxseed increased in the enriched diets, the arachidonic acid content of the yolks increased. Arachidonic acid content was significantly different ($P < 0.05$) in the eggs produced by the hens fed the 5%

flaxseed diet from those fed the other two flaxseed diets and the chia diet. Total ω -6 polyunsaturated fatty acid percentage, calculated as the sum of linoleic and arachidonic acid, was not significantly different ($P > 0.05$) among treatments (Table 4).

The percentage of α -linolenic acid generally increased as the level of dietary chia increased (Table 3). Yolks from hens fed the α -linolenic enriched diets had significantly higher ($P < 0.05$) polyunsaturated α -linolenic acid contents than those from hens fed the control diet. The difference in α -linolenic content was not significant ($P > 0.05$) between the two highest chia diets (T3 and T4), but they both produced egg yolks with a significantly higher ($P < 0.05$) α -linolenic content than T2, the diet having the highest flaxseed content.

The docosahexaenoic (DHA) acid percentage was significantly higher ($P < 0.05$) in the egg yolks produced by the hens fed the highest flaxseed diet (T2) than in the yolks from hens fed all of the other treatments (Table 3).

Total ω -3 polyunsaturated fatty acid percentage, calculated as the sum of α -linolenic and DHA acids, was significantly higher ($P < 0.05$) in the egg yolks produced by hens fed the α -linolenic acid enriched diets than in yolks from hens fed the control diet (Table 4). Comparing the three treatments having any combination of chia and flaxseed that comprised 14% of the diet, the treatment having the highest content of flax (T2) had a significantly lower ($P < 0.05$) total ω -3 content than did either of the other two diets.

Addition of α -linolenic acid to the diets of hens resulted in yolk ω -6: ω -3 and saturated:total ω -3 polyunsaturated

Table 4. Total saturated, monounsaturated, polyunsaturated, polyunsaturated ω -6, polyunsaturated ω -3 fatty acids as well as ω -6: ω -3 and saturated: ω -3 ratios in the yolks

Treatment	Chia (%)	Flax (%)	Saturated	Monounsaturated	Polyunsaturated	Total ω -6	Total ω -3	ω -6/ ω -3	Saturated/ total ω -3
			(% of total fatty acids)						
T0	0	0	33.86	40.05a	22.70c	20.84	1.86d	11.30a	18.32a
T1	7	3	35.09	35.15b	28.52b	20.5	8.18c	2.57b	4.44b
T2	9	5	32.72	30.65c	34.11a	23.18	10.93b	2.13b	3.00c
T3	11.5	2.5	31.46	30.06c	36.10a	23.25	12.85a	1.81b	2.46c
T4	14	0	33.08	29.96c	34.78a	22.11	12.67a	1.76b	2.68c
cr ²			4.29	4.38	4.08	2.94	1.59	1.26	0.8

²Critical range for mean separation.

a–d Means within a column lacking a common letter differ ($P < 0.05$) according to Duncan's multiple range test.

fatty acid ratios that were significantly lower ($P < 0.05$) than those produced with the control diet (Table 4). As the chia content of the diet increased, the ω -6: ω -3 ratios decreased numerically, but were not significantly different ($P > 0.05$). The saturated:total ω -3 polyunsaturated fatty acid ratios in the yolks improved as chia content of the diets increased, with those of the T2, T3 and T4 diets being significantly ($P < 0.05$) lower than yolks produced with the T1 diet.

Sensory Evaluations

Data from the taste panel demonstrated no significant difference ($P > 0.05$) in flavor among treatments (Table 5). Preference was lower for the eggs produced by the hens fed the highest levels of flaxseed (T2). The difference, however, was significant ($P < 0.05$) only from the eggs produced by hens fed the T3 diet.

DISCUSSION

Hen Weight

This study supports the findings of earlier works that used either chia or flaxseed as an ingredient in a hen ration. With chia, body weight was not affected when hens were fed diets having up to 30% chia content (Ayerza and Coates 1999). This agrees with the current study in which body weight was not affected by the chia. Hens fed flax at 5 and 15% of the diet had lower feed intake and lighter body weight than those fed a control diet (Scheideler and Froning 1996). In another study, hens fed flax at 10 and 20% of the diet weighed significantly less ($P < 0.01$) than those fed a control diet (Caston et al. 1994). A negative weight gain was recorded in the current study for the hens fed the T2 diet, the one containing the highest level of flaxseed. Several researchers suggest that the negative effects found with flaxseed when used in poultry rations arise because of the antinutritional cyanogenic glucosides and vitamin B6 antagonist compounds present in flaxseed (Kung and Kummerow 1950; Homer and Schaible 1980; Bell 1989; Bhatti 1993; Bond et al. 1997).

Egg Production, Egg Weight and Yolk Weight

Egg production was not significantly affected by diet. This agrees with Ayamond and Van Elswyk (1995) who reported that a 5% flaxseed diet fed to Single Comb White Leghorn (SCWL) laying hens aged 22 wk produced no significant differences ($P > 0.05$) in egg production from hens fed a

Table 5. The influence of four different chia–flaxseed diets on egg flavor and preference

Treatment	Chia (%)	Flax (%)	Flavor ²	Preference ³
T0	0	0	2.8	3.75ab
T1	7	3	3.1	3.65ab
T2	9	5	2.85	3.10b
T3	11.5	2.5	2.2	4.05a
T4	14	0	3.05	3.70ab
cr ^x			0.94	0.7

²Flavor rating: high intensity, 5; intense, 4; low intensity, 3; very low intensity, 2; normal, 1.

³Preference rating: like very much, 5; like moderately, 4; neither like nor dislike, 3; dislike moderately, 2; dislike very much, 1.

^xCritical range for mean separation.

a, b Means within a column lacking a common letter differ ($P < 0.05$) according to Duncan's multiple range test.

control diet. They did find, however, when hens were fed a 15% flax diet, production declined. Other experiments with flax have shown different results. When DeKalb Delta SCWL laying hens aged 43 wk were fed flaxseed at 5, 10, and 15% of the diet, egg production was greater than for hens fed a control diet (Scheideler and Froning 1996). When a commercial strain of SCWL hens aged 19 wk were fed diets containing 10 or 20% ground flaxseed, egg production was unaffected (Caston et al. 1994). In these experiments, the variation in results may be related to differences in the fatty acid contents of the diets.

No differences were detected in egg weight and yolk weight among treatments. The same finding was reported with laying hens fed 0, 10, 20 and 30% flax diets (Caston and Leeson 1990). Decreasing egg weight and yolk weight over time was reported in another trial, as the level of flax increased in SCWL diets (Caston et al. 1994). Since the strain of laying hens tested in the current trial and those used in the experiments cited above were different, the variation in results could be associated with a strain – diet interaction, as has been reported by Scheideler et al. (1998a).

Cholesterol and Total Lipids

No significant differences ($P > 0.05$) in total lipid and cholesterol contents of the yolks were found between hens fed the α -linolenic acid enriched diets and those fed the control diet. These results are in agreement with other studies where flaxseed was used as the source of the ω -3 acid (Caston and Lesson 1990; Cherian and Sim 1991; Cherian et al. 1995;

Scheideler et al. 1998a) and where lower levels of chia were used (Ayerza and Coates, unpublished data).

Fatty Acid Composition

The fatty acid compositions reported herein are in agreement with a previous trial that used chia in hen rations (Ayerza and Coates 1999), and with those of others that used flaxseed (Caston and Lesson 1990; Cherian and Sim 1991; Cherian et al. 1995; Scheideler et al. 1998a). However, none of these trials used a chia-flaxseed combination to enrich egg yolks.

In general, increasing the α -linolenic acid content in the diet, regardless of its source, produced similar changes in yolk fatty acid content. One exception, however, was arachidonic acid as deposition varied with chia-flaxseed ratio in the diet.

The significant increase in the long chain ω -3 fatty acid docosahexanoic (DHA) found in the yolks with the T2 diet probably arises from desaturation and elongation of α -linolenic acid in the hens' livers, since flaxseed and chia seed lack DHA (Ayerza 1995), as do the other components in the diet. Why this effect was only found with the T2 diet is unknown, but may indicate that flax is better suited to conversion than is chia. As was previously reported (Caston and Lesson 1990; Sim and Jiang 1994; Cherian et al. 1996; Ayerza and Coates 1999), laying hens can convert α -linolenic acid to its elongated metabolite DHA, and deposit it in the yolk. The reason why a significant difference ($P < 0.05$) was found only between the T2 diet and the T3 and T4 diets is not evident based upon available data. It could, however, be related to the chia-flaxseed ratio fed.

Polyunsaturated fatty acids have been reported to limit Δ -9 desaturase activity, which is involved in monounsaturated fatty acid formation (Garg et al. 1988). The present study supports this type of enzyme inhibition, since no significant difference ($P > 0.05$) in yolk monounsaturated fatty acid content was found between T2, T3 and T4 but, with lower polyunsaturated levels in the diets (T0 and T1), monounsaturated levels increased. Also, the results suggest different inhibition effects for chia and flaxseed because, as flaxseed content in the 14% diets increased, monounsaturated levels increased and, as chia levels increased, monounsaturated levels decreased.

The decline in arachidonic acid found in the yolks compared to the control has been reported in other trials when hens were fed diets enriched with α -linolenic acid (Cherian et al. 1995; Cherian and Sim 1991). This has been suggested to be due to the inhibition of the synthesis of this fatty acid when hens receive diets high in α -linolenic acid (Craig-Schmidt et al. 1987).

For the 14% diets (T2, T3 and T4), those with the highest α -linolenic content had the lowest arachidonic acid content in the yolks. This finding can be attributed to an increase in the linoleic: α -linolenic ratio in the diet, and arises because of competition between these two families of fatty acids for Δ 6 and Δ 5 enzymes involved in the processes of desaturation and elongation (Hayek and Reinhart 1998). It has been shown that the ratio of ω -6 to ω -3 fatty acids consumed is

more effective in inhibiting ω -6 fatty acid elongation in other animals than is the absolute amount of ω -3 fatty acid consumed (Boudreau et al. 1991). This ratio effect in terms of linoleic acid deposition is also evident herein, since treatment T1 was significantly different ($P < 0.05$) from T2, T3 or T4, even though T1 had only slightly less α -linolenic acid than the other three treatments.

All of the chia-flaxseed diets produced a significant difference ($P < 0.05$) in ω -6: ω -3 ratios relative to the control diet. A negative relationship between dietary α -linolenic content and yolk ω -6: ω -3 ratio was observed with the 14% diets, with the source of α -linolenic acid also having an effect. The higher the chia content, the lower the ω -6: ω -3 ratio; however, the differences were not statistically significant ($P > 0.05$).

Several studies have shown that an appropriate ω -6: ω -3 ratio must be provided in the human diet. Nutritional recommendations suggest a dietary ω -6: ω -3 fatty acid ratio of 5:1 (Simopoulos 1989; Health and Welfare Canada 1990; British Nutrition Foundation 1992; Food and Agricultural Organization 1994), or 4:1 (Ministry of Health and Welfare of Japan, cited by Okuyama et al. 1997), or even lower (Simopoulos and Robinson 1998). Western diets do not provide these ratios, mainly due to the high ω -6 fatty acid content of the diets consumed (British Nutrition Foundation 1992). The ω -6: ω -3 ratios in the yolks produced by the enriched feeds used in the current trial greatly improve the nutritional quality of the eggs, compared with those of the laying hens fed the control diet.

Addition of chia and/or flaxseed to the hens' diet resulted in lower SFA: ω -3 ratios in the yolks than in those of hens fed the control. No significant differences ($P > 0.05$) among the three highest α -linolenic acid diets were detected; however, a significant difference ($P < 0.05$) between these three diets and the T1 diet was found. This suggests that there is no effect of fatty acid source on SFA: ω -3 ratio. Lowering saturated fatty acid content and raising ω -3 fatty acid levels in the human diet have been reported by several authors to be beneficial in helping to avoid coronary heart disease (Dyerberg et al. 1978; Hennekens et al. 1990; Nettleton 1995). Hence the eggs produced by feeding any of the test diets would provide a healthy alternative as well as a marketing advantage.

Sensory Evaluation

Egg flavor was not significantly different ($P > 0.05$) among diets. This could be related to the lower flaxseed content used than in other studies, or it could be related to the chia-flaxseed combinations fed. The treatment having the highest flaxseed content, T2, had the lowest preference rating, and this was significantly different ($P < 0.05$) from T3.

Other trials have shown that eggs laid by hens fed flaxseed have a characteristic (unpleasant) smell similar to that when hens are fed fish oil (Adam et al. 1989; Jiang et al. 1992; Van Elswyk et al. 1992; Caston et al. 1994; Van Elswyk et al. 1995; Scheideler et al. 1997). A study conducted in five U.S. cities showed that consumers generally are reluctant to buy eggs smelling/tasting of fish (Marshall et al. 1994). The absence of these atypical organoleptic

characteristics in eggs produced by hens fed chia, as was shown in a previous paper (Ayerza and Coates 1999), as well as in this study, represents a significant advantage for this grain compared with flaxseed and marine products and by-products when used as a hen feed.

The taste panel data from this trial suggest it may be possible to include flaxseed up to the levels tested, when mixed with chia, without encountering major differences in consumer preference compared to common eggs. Higher levels of flaxseed, even if mixed with chia, might produce undesirable organoleptic characteristics, as has been found in other trials when feeding flax alone.

CONCLUSIONS

Not all seeds containing α -linolenic acid are biologically equivalent sources that can be used to produce ω -3 enriched eggs. This study showed no advantage to replacing chia with flaxseed when producing ω -3 enriched eggs. In fact, adding flaxseed was found to be disadvantageous. Beyond 3% in the ration, flaxseed adversely affected egg production, egg weight, hen weight gain and egg organoleptic characteristics. Additionally, flaxseed provided lower ω -3 fatty acid deposition in the yolks per unit of α -linolenic acid added to the diet than chia.

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