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Study of the Effects of Dietary Supplementation of Linseeds, Fenugreek Seeds and Tomato-Pepper Mix on Laying Hen's Performances, Egg Yolk Lipids and Antioxidants Profiles and Lipid Oxidation Status

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Abstract

This study aimed at improving the egg quality through cholesterol reduction and enrichment with natural pigments, which could prevent lipids oxidation. Eighty 27 week-old Novogen White laying hens were divided into four groups and given 100 g/hen/d of a standard diet (Control, C), standard diet containing 4.5% ground linseed (Linseed diet, L), linseed diet containing 1% dried tomato and 1% sweet pepper (LTP) or linseed diet containing 2% ground fenugreek (LF). LTP and LF were associated with higher feed consumption ($P<0.05$). Laying rate and feed conversion ratio were not affected ($P>0.05$) by dietary treatment. LF was associated with lower ($P<0.05$) egg weight than L and LTP. All eggs physical characteristics were not affected by dietary treatment ($P>0.05$). Egg yolks carotenoids concentrations in yolks from hens on L and LTP diets were not different ($P>0.05$) and both were higher ($P<0.05$) than those from hens on C and LF. Total phenols concentrations in yolks from hens fed LF were higher ($P<0.05$) than other treatments. Yolks flavonoids concentrations in the LTP and LF groups were not different ($P>0.05$) and both were higher ($P<0.05$) than the C and L groups. Egg yolk triglycerides and total cholesterol concentrations were not affected ($P>0.05$) by dietary treatment. Egg yolks antioxidant activity of the C groups was lower ($P<0.05$) than that of the other groups. Lipids oxidation was also not affected ($P>0.05$). There was a significant enrichment of eggs with carotenoids and an enhancement of their antioxidant status in response to linseed supplementation. There was no beneficial effect of further supplementation with sweet red pepper and dried tomato or fenugreek seed. Further investigations are needed to evaluate the impact of these supplements on fatty acids profile and lipid oxidation status of eggs after their storage.

Keywords: Antioxidant; Carotenoids; Cholesterol; Lipid oxidation; Tomato; Pepper

Introduction

Hen's eggs are an inexpensive and low calorie source of high quality protein and many other nutrients. However, eggs are also a major source of dietary cholesterol that has been frequently incriminated in coronary heart disease [1]. For this reason, many attempts involving dietary manipulation have been made to reduce the egg's cholesterol content [2-4] in addition to its enrichment with ω 3-fatty acids [5,6], minerals, vitamins and carotenoids. The latter are used for both their pigmenting and antioxidant properties [7]. Such named multi-enriched or designer eggs are thought to be more adequate for consumer health than standard eggs. In the recent studies concerned with egg cholesterol reduction, natural plant products like garlic [8] and fenugreek seeds [3,4-10] have been used.

The effect of dietary incorporation of fenugreek seeds on egg cholesterol content has been inconsistent and the reduction was low not exceeding 1 mg/g egg yolk [6]. Eggs fortified with ω 3-fatty acids have been produced through the inclusion of vegetable oil sources in the hen's ration. Because of its high α -linolenic acid content, linseed has been a key feed ingredient for such purpose despite its anti-nutritional effect on layers growth, egg production, egg weight and feed efficiency [11].

Moreover, it was found that long-term use of linseed at an incorporation level of 10% increased the incidence of liver hemorrhages [12] presumably due to the oxidative rancidity of the accumulated long chain unsaturated fatty acids. In addition and of most concern is the fact that eggs produced by hens fed linseed were found to have a fish smell which was thought to arise from their polyunsaturated fatty acids oxidation [13]. To prevent this oxidative rancidity, antioxidants such as vitamin E and butylated hydroxy toluene are added to poultry diet [14]. The protection of polyunsaturated fatty acids from rancidity may be also achieved when adequate amounts of carotenoids and non-carotenoid antioxidants are incorporated into Hen's feed. These antioxidants may be provided as components of the feed's ingredients and or as synthetic or natural supplements

added to the feed, for instance, pepper, tomatoes and fenugreek seeds. These supplements are rich sources of antioxidant compounds like phenolics, flavonoids, carotenoids and vitamins [10,15-17]. These compounds exert their antioxidant activity by scavenging free radical, metal chelation, inhibition of cellular proliferation, modulation of enzymatic activity and signal transduction pathways [18].

Their presence in hen's diet could decrease the susceptibility of cholesterol and lipid oxidation particularly in fatty acids-enriched eggs. It has been reported that tomato powder at 5 or 10 g per kg diet improved egg production persistency, boosted carotenoids and vitamin A contents in egg yolk and reduced yolk lipid oxidation [19]. It has been reported that sweet red pepper exhibits significant abilities in preventing the oxidation of cholesterol or that of docosa-hexaenoic acid during heating [17].

In view of the above, the purpose of the present study was to examine the effects of supplementation of a standard diet based on corn and soybean-meal with linseed, as source of ω 3-fatty acids, with or without fenugreek seeds or tomato-sweet pepper mixture as antioxidant sources on Hen's laying performance, egg yolk lipids and antioxidants profiles and lipid oxidation.

Materials and Methods

Dietary supplements provenance and preparation

Fenugreek seeds (*Trigonella foenum graecum*) and linseeds were purchased from a regional producer located in the region of Mateur (Northeast of Tunisia). Both batches were carefully cleaned from foreign matter then ground through a 0.5 mm sieve. Canned double concentrated tomato paste (5 kg) and sweet pepper powder (1 kg) were purchased from a food market. Tomato paste was dried in a microwave to prevent pigments degradation. For this purpose, tomato paste was first thoroughly blended with ground yellow corn in the ratio 1:2 (w/w) then dried in the microwave (CMI-FR91940, 220-230 V, 1200 W, 20 L, 2450 MHz) for a total of 7 min. During the drying process, the mix was taken out of the microwave at intervals of 1 min, thoroughly mixed and cooled for 1 to 2 min. After drying, the tomato paste-corn mix was cooled at room temperature and stored in an airtight opaque container at room temperature.

Experimental diets preparation

Table 1 Ingredients and chemical composition of the experimental diets.

Ingredients	Diets					
	Control (C)	Linseeds (L)	Linseeds-LTP)	Tomato-Pepper	Linseeds-Fenugreek (LF)	seeds
Linseeds	0	45.0	45.0		45.0	
Fenugreek seeds	0	0	0		20	
Dried tomato	0	0	10		0	
Sweet pepper	0	0	10		0	
Yellow corn	665.0	635.0	615.0		625.0	
Soybean meal	255.0	240.0	240.0		230.0	
Calcium carbonate, Mineral & vitamin mixture	80.0	80.0	80.0		80.0	
Chemical composition (g/Kg, dry matter)						
Crude protein	181.0	180.0	180.0		181.0	
Ether extract	35.6	56.0	52.7		58.8	
Metabolizable Energy [‡] , kcal/kg DM	2750	2850	2830		2860	
α -carotene, $\times 10^{-9}$ g/Kg DM	3.41	5.1	21.7		5.17	
β -carotene, $\times 10^{-9}$ g/Kg DM	3.37	5.36	23.2		5.60	
β -cryptoxanthin, $\times 10^{-9}$ g/Kg DM	3.84	5.50	25.3		5.60	
Lycopene, $\times 10^{-9}$ g/Kg DM	1.77	3.48	15.7		3.12	
Zeaxanthin, $\times 10^{-9}$ g/Kg DM	3.90	5.59	25.7		5.69	
Flavonoids, g CE/Kg DM	2.26	1.59	2.03		8.06	
Total phenols, g GAE/Kg DM	3.02	3.53	2.98		4.48	
Key : [‡] Metabolizable Energy=2707.71 + 58.63EE-16.06NDF [52]						

A standard mash diet (control diet) for laying hens based on corn and soybean-meal was prepared. Thereafter, 3 supplemented diets, designated as follows: linseeds (L), linseeds-tomato-pepper (LTP) and linseeds-fenugreek seeds (LF),

were individually prepared by mixing the control diet thoroughly with the designated supplements at the required incorporation levels as shown in **Table 1**.

Animals and experimental design

Eighty 27 week-old *Novogen White* laying hens (initial live weight=1441 g \pm 76) were divided into 4 homogeneous groups of 20 hens each. Each group was randomly allocated to one of the experimental diets (**Table 1**). In all groups, hens were fed their assigned diet at 100 g/hen/d from the beginning (d 1) to the end of the experiment (d 47). Diets were restricted to 100 g/hen/d to reduce the feed-selection behavior typically observed in laying hens. Feed was offered once daily at 7:30 am and water was provided ad libitum throughout all the trial period. Hens were kept individually in pens in a standard animal room with a 16 h-light/8 h-dark cycle. The temperature in the room was 20 \pm 4°C.

Ethical considerations

All procedures related to animals care, handling, and sampling were conducted under the approval of the Official Animal Care and Use Committee of the Higher School of Agriculture of Mateur (protocol N°05/15) before the initiation of research and followed the Tunisian guidelines.

Measurements and sampling

All hens were weighed individually at the beginning and at the end of the experiment to determine the live weight changes. Egg production and weight were recorded daily and feed refusal was measured weekly. A total of 394 eggs laid during the period from the 26th day to the 34th day of the experimental trial were used for egg physical characteristics measurements (i.e. albumen weight, yolk weight, and shell weight and thickness). During the same period, fresh yolks were daily pooled per two (n=10 samples per dietary treatment). Pooled yolks sampled on the 26th day were used for malondialdehyde (MDA) measurement and those sampled on the 28th day were assayed for reducing power and antioxidant activity. Pooled yolks sampled on the 30th day were used for pigments, total phenolics and total flavonoids and those sampled on the 34th day for lipids profiles determination.

Chemical analyses

The dry matter (DM) content of the diets was determined after drying the samples in an oven at 105°C for 24 h. The other analyses were done on samples dried at 65°C and ground (0.5 mm sieve). Ash content analysis was done by igniting the ground samples at 550°C in a muffle furnace for 10 h. The Association of Official Analytical Chemists method [20] was used for crude protein (CP) determination. Ether Extract (EE) was determined by extraction with diethyl ether for 6 hours in the soxhlet apparatus.

Total cholesterol and triglycerides contents were assessed in fresh egg yolk samples solubilized in 2% (w/v) NaCl solution [21]

employing a standard enzymatic-colorimetric method (cholesterol enzymatic colorimetric test, CHOD-PAP and triglycerides enzymatic colorimetric (GPO-PAP, Biomaghreb, Ibn Khaldoun, Ariana, Tunisia).

Total lipids were gravimetrically determinate on homogenized egg yolk extracted with isopropanol: hexane (30:70, v/v). Total phenols content (TP) was determined according to the Folin-Ciocalteu method as described in Ref. [22]. Total flavonoids content was determined using aluminum chloride method as reported by Patel et al. [23]. Egg yolk (α -carotene, β -carotene, β -cryptoxanthine, lycopene, zeaxanthine) concentrations were determined using the methodology reported [24]. Total antioxidant activity was evaluated by the phosphomolybdenum reducing method as detailed in Ref. [25] and by the ferric reducing assay [26]. Lipid oxidation of fresh egg yolk was estimated by MDA measurement according to the method of Caston et al. [27].

Statistical analysis

Laying performances data were tested for treatment, week and treatment-week interaction effects. Since week and treatment-week interaction were found non-significant ($P>0.05$), only treatment effect was considered. All other data were tested for diet effect only.

The General Linear Model (GLM) procedure of SAS [28] was used according to the following model:

$$Y_{ij} = \mu + a_i + e_{ij}$$

Where:

Y_{ij} =represents the j^{th} observation on the i^{th} treatment

μ =overall mean

a_i =the main effect of the i^{th} treatment

e_{ij} =random error present in the j^{th} observation on the i^{th} treatment.

All reported data were reduced to, at most, three significant digits.

Results

Laying performance and egg physical characteristics

Laying performance and egg physical characteristics are shown in **Table 2**. Daily feed refusal and consumption were both affected by dietary treatment ($P<0.0001$). Both LTP and LF diets were associated with lower feed refusal and, consequently, with higher intake. Hen's body weight loss at the end of the experimental trial, laying rate and feed conversion ratio (FCR) were not affected ($P>0.05$) by dietary treatment. Compared with the control (C), the L and LTP diets were associated with higher ($P<0.05$) egg weight. LF was associated with lower ($P<0.0001$) egg weight and egg mass than LTP and lower egg weight than L. Egg physical characteristics (shell weight, shell thickness,

albumen weight, yolk weight) were not affected by dietary treatment ($P>0.05$).

Table 2 Hens' live weight changes, laying performances and egg physical characteristics according to fed diets.

	Diets				SEM [†]	Pr
	C	L	LTP	LF		
Live weight change ^a , g/47d	-66.5	-65.2	-85.3	-103	12.1	NS
Refusal ^a , g DM/hen/d	1.35 ^a	1.22 ^a	0.39 ^b	0.41 ^b	0.18	***
Feed Consumption ^a , g DM/hen/d	88.5 ^b	89.0 ^b	89.8 ^a	89.8 ^a	0.18	***
Laying rate ^a , %	97.6	96	98.2	98	0.69	NS
Egg weight ^a , g	55.9 ^b	57.6 ^a	57.3 ^a	56.0 ^b	0.22	***
Egg mass ^a , g/hen/d	54.5 ^b	55.2 ^{ab}	56.2 ^a	54.9 ^b	0.44	*
Feed Conversion ratio ^a	1.65	1.65	1.6	1.64	0.03	NS
Shell weight ^δ , g	5.41	6.15	5.89	5.65	0.25	NS
Shell thickness ^δ , mm	0.39	0.4	0.42	0.41	0.008	NS
Albumen weight ^ε , g	33.9	35.5	34.7	33.8	0.61	NS
Yolkweight ^{εδ} , g	13.8	13.8	13.9	13.8	0.22	NS

Key: Control=diet unsupplemented, L=diet supplemented with ground linseeds at 4.5%, LTP=diet supplemented with ground linseeds (4.5%), dried tomato (1%) and sweet pepper powder (1%) mix, LF=diet supplemented with ground linseeds (4.5%) and ground fenugreek seeds (2%) mix, α: determined through the whole experimental period(47 days, 3663 eggs), δ: determined on eggs of the 26th-34thdays of the experimental trial (N=394 eggs), ε: yolk without vitelline membrane; SEM=standard error of the mean;***=p < 0.0001, **=p<0.001, *=p<0.05, NS=p>0.05, a; b: Means within the same raw with different superscripts letters are significantly different (p<0.05).

Egg yolk antioxidants concentration

Egg yolk antioxidants (α-carotene, β-carotene, β-cryptoxanthine, lycopene, zeaxanthine, total phenols and flavonoids) concentrations are shown in **Table 3**. Concentrations of all yolk antioxidants significantly differed among dietary

treatments. Carotenoids concentrations (α-carotene, β-carotene, β-cryptoxanthine, lycopene, zeaxanthine) in yolks from hens fed L and LTP were not different ($P>0.05$), but both were higher ($P<0.05$) than those from hens that consumed C and LF.

Table 3 Egg yolk antioxidant compounds concentrations.

	Diets				SEM [†]	Pr
	C	L	LTP	LF		
α-carotene, μg/g	11.0 ^b	12.3 ^a	12.7 ^a	10.3 ^b	0.37	**
β-carotene, μg/g	11.2 ^b	12.3 ^a	12.9 ^a	10.5 ^b	0.36	***
β-Cryptoxanthine, μg/g	12.1 ^b	13.6 ^a	14.2 ^a	11.6 ^b	0.39	***
Lycopene, μg/g	7.66 ^b	8.41 ^a	8.90 ^a	7.06 ^b	0.25	***
Zeaxanthine, μg/g	12.4 ^b	13.8 ^a	14.4 ^a	11.8 ^b	0.4	***
Total phenols, mg GAE/g [‡]	1.86 ^c	2.17 ^b	2.16 ^d	2.19 ^a	0.09	*
Flavonoids, mg CE/g [‡]	1.91 ^b	1.53 ^b	2.96 ^a	3.02 ^a	0.28	***

Key: Control=diet unsupplemented, L=diet supplemented with ground linseeds at 4.5%, LTP=diet supplemented with ground linseeds (4.5%), dried tomato (1%) and sweet pepper powder (1%) mix, LF=diet supplemented with ground linseeds (4.5%) and ground fenugreek seeds (2%) mix, SEM[†]=standard error of the mean;
[‡]Total phenols expressed in mg gallic acid equivalent, mg GAE/g
[‡]Flavonoids expressed in mg catechin equivalent, mg CE/g
 ***=P<0.0001, **=P<0.001, *=P<0.05, NS=P>0.05
 a,b: Means within the same raw with different superscripts letters are significantly different (P<0.05)

Total phenols concentrations in yolks from hens on LF were higher ($P<0.05$) than those from hens that received L, LTP and C.

Flavonoids concentrations in yolks from hens on LTP and LF were statistically similar ($P>0.05$) and both were higher ($P<0.05$) than those from hens on C and L.

Egg yolk lipids profile and oxidative status

Egg yolk total lipids, triglycerides (TG) and total cholesterol are shown in **Table 4**. Only total lipids concentrations were affected ($P < 0.05$) by dietary treatment.

Table 4 Fresh egg yolk lipids profile and oxidative status.

	Diets				SEM ^Y	Pr
	C	L	LTP	LF		
Total lipids, %	32.8 ^a	33.6 ^a	31.7 ^b	31.8 ^b	0.31	***
Triglycerides, mg/g	172	175	178	178	4.24	NS
Cholesterol, mg/g	15.3	15.2	14.7	15.2	0.25	NS
Antioxidant activity, mg AAE/g [§]	4.48 ^b	5.07 ^a	5.16 ^a	5.18 ^a	0.13	*
Antioxidant activity, mg GAE/g [‡]	3.79	4.38	4.14	3.15	0.36	NS
Thiobarbituric acid reactive substances (TBARS), µg MDA/g	0.11	0.14	0.15	0.16	0.017	NS

Key: Control=diet unsupplemented, L=diet supplemented with ground linseeds at 4.5%, LTP=diet supplemented with ground linseeds (4.5%), dried tomato (1%) and sweet pepper powder (1%) mix, LF=diet supplemented with ground linseeds (4.5%) and ground fenugreek seeds (2%) mix, SEM^Y=standard error of the mean;
[§]Antioxidant activity evaluated as phosphomolybdenum reducing power and expressed in ascorbic acid equivalent (AAE)
[‡]Antioxidant activity evaluated as ferric reducing power and expressed in gallic acid equivalent (GAE)
 ***= $P < 0.0001$, **= $P < 0.001$, *= $P < 0.05$, NS= $P > 0.05$
 a,b: Means within the same raw with different superscripts letters are significantly different ($P < 0.05$).

Total lipids concentration values were not different ($P > 0.05$) between yolks from hens on C (32.8%) and L (33.6%) as well as between yolks from hens on LTP (31.7%) and L (31.8%). Egg yolk triglycerides concentrations ranged from 172.4 to 178.2 mg/g and cholesterol concentrations ranged from 14.7 to 15.3 mg/g. **Table 4** shows also antioxidant activity and oxidative stability traits of egg yolk. Only antioxidant activity measured by the phosphomolybdenum reducing method (reducing power) was affected ($P < 0.05$) by dietary treatment. This parameter was lower ($P < 0.05$) in yolks from hens fed on C (4.48 mg ascorbic acid equivalent/g) than in those from hens that consumed L, LTP or LF. These dietary treatments were associated with similar antioxidant activity ranging from 5.04 to 5.18 mg ascorbic acid equivalent/g. Antioxidant activity measured by the ferric reducing method was similar for all the dietary treatments and ranged from 3.15 to 4.38 mg gallic acid equivalent/g. Lipids oxidation values, measured by the MDA measurement method, were comparable between the four dietary treatments and ranged from 0.11 to 0.16 µg MDA/g yolk.

Discussion

Effects of linseed supplementation

Currently, linseed is often used in the production of ω -3-enriched eggs and some reports indicated that there was an increase in egg yolk unsaturated fatty acids concentrations when hens were fed yellow corn-soybean meal diets containing linseed at an incorporation rate of 5% [29-31]. In the present study, linseed was used for ω -3-fatty acids enrichment at an inclusion level of 4.5% of the diet to avoid its potential negative impact on Hen's production performance and egg smell and taste. At this level, it increased diet's ether extract from 3.56 to 5.6% DM and estimated metabolizable energy by 100 kcal/kg

DM. It had a moderate effect on diet's carotenoids, total phenols and flavonoids concentrations. Unfortunately, its effect on diet's and eggs fatty acids profiles was not determined. Linseed inclusion was without impact on feed consumption. Current literature is conflicting with regard to the effect of linseed dietary incorporation on feed consumption. Bean and Leeson [12] and Scedeler and Froning [30] reported a decrease in feed intake, whereas Yassein et al. [29], Caston and Leeson [32] and Novak and Sheideler [33] reported the opposite finding. In the present study, feeds offered were restricted to 100 g/d/hen and linseed was included at 4.5% level, which may explain the discrepancy with the findings reported above. Despite feed restriction, Hen's live body weight losses were low (65 vs. 66.6 g/47 d) and laying rates were high (97.6 vs. 96%) and not affected by linseed inclusion. Furthermore, only egg weight was significantly increased from 55.9 to 57.6 g/egg. This increase was, however, without significant impact on egg mass and FCR as well as on egg physical characteristics (shell, albumen and yolk weights). These results agreed partially with those reported by Yassein et al. [29] who found that linseed used at the 5% dietary incorporation level resulted in significant increase in laying rate and egg mass. However, this dietary supplementation was without effect on egg weight, FCR and, albumen, yolk and shell percentages.

In addition, although the concentrations of the different carotenoids in the linseed-supplemented diet (L) were moderately higher than those assayed in the control diet (C), there was a significant enrichment of egg yolk in each of these pigments. This enrichment was observed even though carotenoids concentrations in egg yolk from hens on the control diet were higher than those (1.07 and 7.09 µg/kg for β -carotene and zeaxanthin, respectively) reported by Kotrbacek et al. [34] for diet containing 33% wheat, 30% corn and 24% soybean meal.

This difference may be attributed to the difference between the ingredient composition of both diets, particularly, regarding the proportion of corn. To the best of the authors knowledge, data on carotenoids levels in egg yolk in response to linseed dietary supplementation are lacking. However, Yassein et al. [29] reported a decrease in yolk color due to linseed supplementation at a level of 5%, which may be assumed as a reduction in yolk carotenes concentrations.

The control diet was associated with a total phenols concentration (3.02 mg GAE/g) much higher than that (0.54 mg GAE/g DM) reported by Amar et al. [7].

The results obtained from the yolk's flavonoids concentration measurements revealed that the dietary inclusion of linseed failed to increase this parameter. It is difficult to compare this finding with those of other studies because in the literature, works with regard to this aspect are lacking. Concerning egg yolk lipids profile, antioxidant activity and lipids oxidation status, our results showed that the control diet resulted in total lipids (32.8%), triglycerides (172 mg/g yolk) and cholesterol (15.3 mg/g yolk) concentrations within the ranges of values observed in conventional eggs. The MDA concentration (0.11 μ g/g yolk), which reflects yolk lipids oxidation, was lower than that (0.7 μ g MDA/g yolk) reported by Venglovska et al. [35] for a diet containing 33.5% wheat, 31% corn and 24.5% soybean meal.

This low MDA concentration may be explained firstly by the fact that this parameter was measured on fresh eggs and secondarily by the high concentrations of carotenoids, phenols and flavonoids in egg yolk. Indeed, antioxidant activity of these compounds was demonstrated by their phosphomolybdenum and ferric reducing powers, which were equivalent to 4.48 mg ascorbic acid/g yolk and 3.79 mg gallic acid/g yolk, respectively. Linseed supplementation had no effect on total lipids, triglycerides, cholesterol and MDA concentrations in egg yolk and increased its power to reduce phosphomolybdenum (equivalent to 5.07 mg ascorbic acid/g yolk). This improvement may be attributed to the observed increase of carotenoids concentrations in yolk. The results of the present study are in partial agreement with those found by Imran et al. [36] who reported no significant increase or decrease of the total lipids and cholesterol contents in egg yolk in response to the dietary inclusion of extruded linseed at 10%, 20% and 30% levels. It has been showed that yolk cholesterol concentration was not affected noticeably by linseed level in the diet [30-38]. Nevertheless, Basmacioglu et al. [31] reported that linseed inclusion decreased yolk cholesterol concentration at a level of 8.64% of the diet but it had no effect at 4.32% level. Studies with regard to the effect of linseed on egg antioxidant status are scarce in laying hens. Chen et al. [39] evaluated the effects of geese's diet supplemented with linseed on the antioxidant status of their offspring and found that the MDA content in the livers of goslings decreased as linseed supplementation levels increased. This finding suggests that better antioxidant status of the eggs from hens on linseed-supplemented diets could be achieved. In summary, although response to linseed supplementation in terms of fatty acids profile was not evaluated, there was a significant enrichment of eggs with carotenoids and an enhancement of their antioxidant status.

Effects of sweet pepper and dried tomato supplementation

Tomato and red pepper are natural sources of antioxidants such as ascorbic acid, tocopherols, phenolics, flavonoids and carotenoids. Carotenoids in tomato comprise much more lycopene than β -carotene. Lycopene is largely responsible for the red color and has been found to exhibit a cholesterol-lowering effect in human beings [40]. Carotenoids in sweet red pepper contain more β -carotene than lycopene. They include, also, other specific carotenoids namely capsantin and capsorubin [41]. Thus, diets supplemented with both tomato and pepper could provide a wider array of antioxidants than diets enriched with one supplement.

In the present study, sweet red pepper and dried tomato were included together in the diet at a level of 1% each to serve as complementary sources of antioxidants. These antioxidants could be transferred into the egg yolk and apart from their coloring effect, which is not discussed herein; they could prevent yolk lipids oxidation particularly when they are incorporated into linseed supplemented diet. Their inclusion did not change the diet gross chemical composition, total phenols and total flavonoids concentrations and was associated to a 4 to 5-fold increase of all assayed pigments (L vs. LTP in **Table 1**). However, feed refusal was reduced and consequently feed consumption was increased from 89.0 to 89.8 g DM/hen/d without affecting laying parameters and egg physical traits. Furthermore, the sweet pepper and dried tomato addition to the feed decreased the egg yolk total phenols concentration from 2.17 to 2.16 mg GAE/g, but it increased flavonoids concentration from 1.53 to 2.96 mg CAE/g. Concentrations of the other yolk antioxidants compounds only increased slightly.

This supplementation also decreased the egg yolk total lipids concentration from 33.6 to 31.7%. However, it had no effect on oxidative status traits. This inefficiency of feed pigments transfer to the egg yolk suggests that there was no need to use pigment concentrations higher than those provided by the linseed-supplemented diet that ranged from 3.48 μ g lycopene/g DM to 15.7 μ g lycopene/g DM. In this respect, diets containing 1.0 to 4.2 μ g zeaxanthin/g DM resulted in 4.0 to 9.9 μ g zeaxanthin/g DM egg yolk [42]. Moreover, Jiang et al. [43] found that feeding hens with 200 mg β -carotene/Kg resulted in an egg yolk content of β -carotene equal to 5.2 mg/Kg.

Studies on combined tomato and pepper supplementation in laying hens are lacking. Many reports investigated the feasibility of feeding tomato or tomato waste and their results were inconsistent. The cause of divergence could be due to a number of factors such as tomato fraction used and its inclusion rate, the ingredient composition of the supplemented diets as well as the response traits. In this regard, Akdemir et al. [19] found that tomato powder added at 0.5 and 1% enhanced linearly laying performance and increased concentrations of egg yolk lycopene, β -carotene, and lutein and decreased MDA concentrations. Habanabashaka et al. [44] reported that tomato waste meal can be included in layer diets up to 6% without any adverse effect on egg production rate. This inclusion level enhanced yolk lycopene concentration and reduced its cholesterol content. It has been

found that dried tomato pomace incorporated into the diet at up to 19% was without adverse effect on laying performance and egg physical traits [45]. Moreover, including dried tomato peel at up to 130g/kg DM of feed decreased egg yolk cholesterol and increased total phenols, β -carotene and lycopene [7].

With regard to pepper dietary supplementation effect, red pepper pigment used at levels of 0.3, 0.6, 1.2, 2.0, 4.8 or 9.6 ppm and red pepper powder used at level of 0.8% did not affect egg-laying performance, feed consumption and FCR but increased the yolk color score [46]. Similar effects were observed when three kinds of red pepper were included at level of 0.5% [47].

In summary, the enrichment of eggs with carotenoids and the enhancement of their antioxidant status attributed to linseed supplementation were not improved in response to further supplementation with sweet red pepper and dried tomato.

Effects of fenugreek seed supplementation

Fenugreek seed has been used in many studies in attempt to reduce egg cholesterol content and the response has been inconsistent. However, it contains many bioactive compounds including powerful antioxidants that could prevent lipids oxidation, particularly in eggs enriched with polyunsaturated fatty acids.

In the present study, in order to reevaluate its effect on egg cholesterol content and investigate its impact on lipids oxidative status, fenugreek seed was included at 2% into linseed-supplemented diet. Non-supplemented and supplemented diets had similar gross chemical composition, pigments concentrations and a 4 to 5-fold difference in flavonoids concentrations (L vs. LF in **Table 1**). Fenugreek seed supplementation reduced feed refusal and consequently increased feed consumption but these statistical significant changes corresponded to a difference of only 0.8 g DM/hen/d resulting in similar laying performance and egg physical traits except egg weight which was reduced from 57.6 to 56.5 g. With respect to feed consumption, some studies reported a negative effect [9,47] and others reported no effect [4,48-50].

Likewise, contradicting effects on egg production rate and egg physical traits have been reported. Abdouli et al. [4] found no effect when ground fenugreek seed was added at 2 to 6 g/hen/d. By contrast, Nasra et al. [9] reported an increase in egg production rate and egg mass for hens that received feed containing 0.1 or 0.5% ground fenugreek seeds, and a decrease in egg weight for hens fed on 0.5% ground fenugreek seeds. Moustafa [50] found a significant increase in egg production rate and egg mass when fenugreek seed were included in the diet at level of 0.05 or 0.15 %. The authors observed an increase in egg weight only when fenugreek seed inclusion level was of 0.05%. In this study, pigments concentrations were similar in both diets (L and LF), yet supplementation reduced pigments concentrations in egg yolk suggesting either a reduction of their absorption or an increase of their metabolism resulting in low transfer into the egg yolk. Reports on the effect of fenugreek seed supplementation on egg yolk pigmentation are lacking. Indirectly, on the bases of egg yolk color scores, Abdouli et al.

[51] reported no effect of fenugreek seed supplementation at 2 g/hen/d. Total lipids concentration decreased from 33.6 to 31.8% and triglycerides and cholesterol concentrations were not affected. This inefficacy to reduce egg yolk cholesterol was in line with some findings [4,52] and not with others [3,9,50]. Egg yolk total phenols concentration which increased from 2.17 to 2.19 mg GAE/g and egg yolk flavonoids concentration which increased from 1.53 to 3.02 mg CE/g in response to their high concentrations in fenugreek seed supplemented diet (LF) did not change yolk antioxidants concentrations measured by phosphomolybdenum and ferric deduction as well as lipids oxidation expressed in terms of MDA concentration.

These results might be explained by the fact that these traits were determined on fresh eggs. Their determination on stored eggs would have shown an enhanced lipid oxidation status.

Conclusion

In conclusion, although response to dietary incorporation of linseed in terms of fatty acids profile was not evaluated, there was a significant enrichment of eggs with carotenoids and an enhancement of their antioxidant status. This beneficial response was not improved by a further dietary supplementation with sweet red pepper and dried tomato or fenugreek seed. Further investigations are needed to evaluate the impact of these supplements on fatty acids profile and lipid oxidation status of eggs after their storage.

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