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Effects of dietary supplementation of palm fatty acid powders on performance, internal egg quality and yolk oxidation stability in laying hens during early egg production

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ABSTRACT

The present study was conducted with Hy-Line W-36 Leghorn hens to investigate the effects of dietary substitution of soybean oil (SO) with 2 commercial palm fatty acid powders (PFAP) on performance, egg quality measurements and oxidative stability of yolk lipids. Laying hens (225) were fed with a basal diet containing 3.5% soybean oil in which SO was replaced with PFAP (either energizer A or B) by 25, 50, 75 or 100%. Dietary treatments had no significant effect on egg production during 27 to 32 wk of age, but incremental levels of both PFAP sources reduced hen-day egg production during 32 to 37 wk of age. Energizer B was associated with more egg production and egg mass compared with energizer A. Dietary supplementation with PFAP increased feed intake compared with control group during 32 to 37 wk of age. Although feed conversion ratio (FCR) was not affected by experimental diets from 27 to 32 wk of age, the best FCR values assigned to the birds on control diet in the second 35 d, and birds on energizer B improved FCR compared with those on energizer A-diets. PFAP improved Haugh unit during both recording periods. All dietary replacement levels of PFAP were associated with higher standing-up quality of egg yolks. Diets supplemented with energizer B improved shell thickness and subsequently eggshell breaking strength compared with energizer A or control diets. Yolk cholesterol content was higher in eggs laid by hens fed on A- and B-containing diets compared with control birds. Dietary inclusion of PFAP partially or completely in place of SO reduced egg yolk malondialdehyde. The present findings suggested that one can replace 75% of SO with PFAP without any detrimental effect on performance and egg quality. In addition, yolk oxidative stability could be improved with PFAP.

Key words: Laying hens, Palm fatty acid, Shell strength, Yolk oxidation stability

Fat reduces the feed passage rate through the gastrointestinal tract allowing a better absorption of all nutrients present in diet (Baiao and Lara 2005). Fat in diets fulfil high energy requirements of rapidly growing broilers and high egg-producing hens (NRC 1994, Korver *et al.* 1998, Isika *et al.* 2001). Addition of fat in broiler breeder diets, without increasing ME intake, increased egg production and feed conversion efficiency (Brake 1990).

The lipid composition of poultry eggs is an area of primary consumer concern due to the close connection between certain dietary lipids and the development of coronary heart disease and some forms of cancer (Chow 1992, Haraldsdóttir 1999, Hartman and Wilhelmson 2001). Efforts are centered on reducing the cholesterol content of the final products (Cherian *et al.* 2002). It is possible to modify the fatty acid profile of the yolk by dietary alterations of the lipid sources in laying hen's diet (Grobas *et al.* 2001, Pardio *et al.* 2005). Mostly soybean and corn oils, rich in n-6 PUFA (Simopoulos and Robinson 1998, Pardio *et al.* 2005), are added in commercial poultry diets but these are expensive and compete with human nutrition. Palm oil (PO), an edible plant oil, accounts for about one quarter of the world's fats and oils supply (Khosla 2006) and is expected to surpass SO in less than a decade (FAO 2007). Crude PO contains approximately an equal amount of saturated (SFA) and unsaturated fatty acids (USFA) with palmitic acid, making the highest (more than 45%) proportion (Khosla 2006). PO or mixtures of palm oil might replace animal fats without any negative impact on carcass quality (Rodriguéz *et al.* 2002). However, reports on this dietary utilization of palm oil FA compared to other vegetable oils or animal fats in poultry feeds are limited. The objective of the study was to provide some information on the utilization capacity of PO fatty acids in layer diets with concern to their effects on laying performance, egg quality and yolk oxidation stability.

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MATERIALS AND METHODS

Birds, husbandry and experimental procedures

All experimental procedures were approved by the Animal Use and Care Committee of the Isfahan University of Technology. Single Comb White Leghorn hens (225) at 25 wk of age were randomly assigned to the 9 dietary treatment groups (5 cage replicates of 5 hens each). The birds were housed in layer wire-floored cages at a density of 405 cm^2 per bird in a windowless house, and were given artificial light (16L: 8D) throughout the duration of study. *Ad lib.* diets and water were offered. Standard management rules were followed.

The experimental treatments included a corn-soybean meal control diet containing 3.5% SO (Table 1) in which oil source was replaced with each of 2 commercial fatty acid powders of palm oil (Energizer A as granulated fat powder and Energizer B as Ca salt of FA) by 25, 50, 75, or 100%. The trial lasted for 2 periods of 35-d (5 wk) and commenced after a 14-d pre-experimental (adaptation) period in which each hen received its respective experimental diet. Dietary soy-oil (SO) was replaced by palm fatty acid powders (PFAP) irrespective of their ME values, but all of the diets were similar for other nutrients (Hy-Line International 2007), and exceeded the NRC (1994) recommendations for laying hens requirements.

Hen-day egg production was measured daily throughout the main experimental periods. All produced eggs were saved and weighed daily, and weights were recorded per each cage replicate. Feed consumption and body weight of the hens were measured at the beginning and at the end of each 35-d (wks of 32 and 37 of age) experimental period. The 10 eggs produced per replicate cage at the final day of each experimental period were stored to analysis for yolk cholesterol, triglycerides, and malondialdehyde (MDA) could be performed.

Egg quality parameters were evaluated every 35-d. All eggs which were laid during the last 3-d of each period was collected and egg internal (yolk colour, Haugh unit, and yolk index) and external (eggshell thickness and shell breaking strength) qualities were measured as given below. Each egg was weighed and broken (after measuring eggshell breaking strength) onto a flat surface. The height of the albumen (average of 4 points), mid-way between the yolk and the edge of the thick albumen, was measured with a tripod micrometer. Haugh Units were calculated as per Eisen *et al.* (1962) and Silversides (1994). The yolk index was obtained by dividing the height of the yolk by its diameter. Visual yolk colour was measured by the Roche yolk color fan (Vuilleumier 1969). Egg shell thickness was determined by micrometer screw gauge.

Chemical analysis of diets and eggs

The diets were analyzed according to AOAC (2002) for

Table 1. Ingredients and nutrient composition of basal experimental diets during 27 to 37 wks of age

Item (% unless stated otherwise)	27 to 32 wk of age	32 to 37 wk of age
Corn, yellow	51.95	50.01
Wheat	3.05	6.45
Soybean meal, 44% CP	23.51	22.11
Alfalfa meal, 17% CP	2.00	2.00
Wheat bran	3.00	3.00
Soybean oil[1]	3.50	3.50
Limestone	4.37	4.40
Oyster shell	5.00	5.00
Dicalcium phosphate	1.80	1.75
Mineral premix[2]	0.25	0.25
Vitamin premix[3]	0.25	0.25
Zeolite	0.50	0.50
Sodium chloride	0.25	0.25
Na bicarbonate	0.20	0.20
Na sulphate	0.10	0.10
DL-methionine	0.19	0.17
L-lysine. HCl	0.08	0.06
Analyzed nutrient composition[4]		
AME_n (kcal/kg)	2800	2800
Crude protein	16.50	16.10
Ether extract	6.16	6.12
Methionine	0.44	0.42
Methionine + cysteine	0.71	0.68
Lysine	0.90	0.85
Calcium	4.00	4.00
Non-phytate P	0.43	0.42
Sodium	0.18	0.18

[1]Soybean oil in control diet was replaced by each of two palm oil fatty acid powders (energizer A as granulated FAT powder and energizer B as Ca salt of FA) by 25, 50, 75 or 100% to provide 9 dietary treatments.

[2]Provided per kilogram of diet: Mn (from $MnSO_4.H_2O$), 70 mg; Zn (from ZnO), 60 mg; Fe (from $FeSO_4.7H_2O$), 50 mg; Cu (from $CuSO_4.5H_2O$), 8 mg; I (from $Ca(IO_3)_2.H_2O$), 0.86 mg; Se, 0.30 mg.

[3]Provided per kilogram of diet: vitamin A (from vitamin A acetate), 8700 IU; cholecalciferol, 2500 IU; vitamin E (from DL-α-tocopheryl acetate), 16 IU; vitamin B_{12}, 0.03 mg; riboflavin, 2.6 mg; niacin, 34 mg; calcium pantothenate, 35 mg; menadione (from menadione dimethyl-pyrimidinol), 1.50 mg; folic acid, 0.60 mg; thiamine, 3 mg; pyridoxine, 2.50 mg; biotin, 30 mg; ethoxyquin, 125 mg.

[4]Nutrient composition reported is based on the presence of soybean oil. Metabolizable energy content and dietary ether extract are certainly different between the experimental diets. Diet AME_n values were calculated based on measured nutrient composition by using NRC (1994) recommended formula.

moisture, crude protein, ether extract, crude fiber, and ash. All of the samples were analyzed in triplicate.

Total yolk lipids (10 eggs per cage at 31 wk of age) were extracted as per Folch *et al.* (1957). The yolk cholesterol was analyzed according to Jiang *et al.* (1991) and Hamil and Soliman (1994). Egg yolk triglycerides and cholesterol were analyzed with a biochemical analyzer.

Frequently, primary (lipid hydroperoxides) and secondary (thiobarbituric acid reactive substances; TBARS) oxidation products are measured as an index for oxidative stability of eggs and egg products (Cherian *et al.* 1996, Galobart *et al.* 2001). TBARS values (expressed as n mol of MDA per gram of yolk) from egg yolk were determined (Dorman *et al.* 1995) using 1,1,3,3- tetraethoxypropane as standard.

Statistical analysis

The data on productive performance and egg quality measurements were analyzed using SAS (SAS Institute 1999) based on a completely randomized design. Contrast comparisons were performed to evaluate control birds versus other dietary treatments, as well as to compare replacement levels or fat sources (energizer A vs. energizer B). The differences between treatments were determined using least significance difference test. The data on both performance and egg internal and external qualities are expressed as 2 individual 35-d periods (27 to 32 and 32 to 37 wk).

RESULTS AND DISCUSSION

Dietary treatments had no significant effect (Table 2) on egg production during the first 35-d period (27 to 32 wk). During 32 to 37 wk of age, however, the significant differences (P<0.001) were found among dietary treatments for egg production performance. Increasing replacement level of PFAP in place of SO caused a significant (P<0.001) reduction of laying performance as measured by hen-day egg production. This decline was more pronounced (P<0.05) with energizer A-diets compared with energizer B-supplemented ones. Similarly, egg mass was also superior in B-supplemented diets than that of A-groups during both 27 to 32 (P<0.05) and 32 to 37 (P<0.01) wk of age. Increasing dietary inclusion level of A in replacement with SO resulted in a significant (P<0.001) decrease in egg mass.

A high dietary content of FFA may decrease the energy value (or utilization) of the fat (Wiseman and Salvador 1991, Blanch *et al.* 1995) as evidenced in the present study with 75 or 100% PFAP-supplemented diets. The main reason for this apparent discrepancy is a reduced emulsification (and therefore hydrolysis and absorption) of the dietary fat, because of the secretion of the emulsifying bile acids is induced by the presence of triglycerides and 2-monoglycerides not FFA in the small intestine (Sklan 1979). Furthermore, diets rich in FFA content need more bile salts for efficient absorption. Although this fact may be a reason

Table 2. Effects of dietary replacement of soybean oil with two commercial palm fat sources on laying hen performance during 27 to 37 wks of age

Fat source[1] and replacement level (%)		Soy oil	Egg production (%)		Egg mass (g/d per hen)		Feed intake (g/d per hen)		FCR (g feed/ g egg)	
			27 to 32 wk	32 to 37 wk	27 to 32 wk	32 to 37 wk	27 to 32 wk	32 to 37 wk	27 to 32 wk	32 to 37 wk
Control		100	93.11	91.16	54.17	52.31	95.03	93.61	1.76	1.79
A	25	75	93.56	91.16	54.26	52.96	95.24	96.23	1.76	1.82
	50	50	91.78	88.00	54.95	52.56	97.88	98.47	1.78	1.87
	75	25	91.78	85.89	53.68	50.50	93.77	94.48	1.75	1.87
	100	0	91.56	85.05	53.27	49.95	96.05	94.94	1.80	1.90
B	25	75	92.67	91.58	55.40	54.86	97.07	97.53	1.75	1.78
	50	50	93.78	90.32	55.56	53.34	97.56	97.96	1.76	1.84
	75	25	92.44	87.79	55.30	51.97	98.29	94.07	1.78	1.81
	100	0	91.33	88.84	54.03	52.54	97.10	97.61	1.80	1.86
			Probability							
Treatment			NS	***	NS	***	**	**	NS	***
Contrasts										
Control vs. others			NS	0.0749	NS	NS	NS	*	NS	*
Replacement level			NS	NS	NS	***	NS	***	NS	**
A vs. B			NS	*	*	**	**	NS	NS	**
100% of 3 sources			NS	***	NS	*	NS	*	NS	**
Pooled SEM			0.9910	1.0133	0.6525	0.5481	0.8554	0.9607	0.0213	0.0188

[1]Fat sources were used in place of soybean oil in control diet, were fatty acid powders of palm oil (energizer A and energizer B). NS: not significant; *P<0.05; **P<0.01; ***P<0.001.

for decreased laying performance in the highest levels of dietary palm fatty acids, but it appears that the influence of FFA inclusion into the diets to be of minor importance and confined to younger birds (Wiseman and Salvador 1991). The negative effects of higher levels of PFAP supplementation on laying performance is mainly related to saturation of FFA contained in commercial PFAP used in this study. Energizer A was analyzed to be contained in 86.12% palmitic acid (Jahanian, unpublished data). Compared with A, Energizer B consisted of higher oleic acid (35.09 vs. 7.05%) and lower palmitic acid (48.15 vs. 86.12%) content (Jahanian, unpublished data). The weak egg production performance at the highest levels of A substitution (75 and 100%) may be attributed to the higher amounts of SFA contained in PFAP compared with the most vegetable oils. The harmful effects of saturated free fatty acids on laying hen's performance could be explained by the fact that energizer B (more unsaturated profile) had less detrimental impact on egg production in the herein study. Consistent with our conclusion, Vila and Esteve-Garcia (1996) found that in 3-wk-old broilers, dietary saturated FFA decreased the digestibility and metabolizable energy value of the supplemental fat, while dietary unsaturated FFA did not.

The birds fed on energizer B-diets had more egg production and higher egg mass than in A-fed groups. Probably, feeding of free fatty acids caused a reaction between carboxylic group of FA and ionized minerals such as Ca forming soaps. If these soaps are insoluble, they diminish energy value of fat. It is known that the Ca soaps of USFA are more absorbable than Ca soaps of SFA. This fact may be a reasonable explanation why hens on energizer B-supplemented diets performed better than those on energizer A-diets. Our findings are in agreement with Atteh and Leeson (1984, 1985). Therefore, while some soaps were inevitably excreted in the feces when high amounts of Ca were fed, dietary USFA had only negligible effects on the ME value of dietary fat and no effects on mineral retention and subsequently eggshell quality. Moreover, Atteh and Leeson (1985) suggested that the Ca soaps are probably formed in the large intestines of the mature birds, where they cannot interfere with energy and nutrient utilization.

Birds on energizer B-supplemented diets consumed ($P<0.01$) more feed than those on energizer A or control diets during the first 35-d period; during the period of 32 to 37 wk of age, however, the groups fed different levels of PFAP had higher ($P<0.05$) feed intake than control group. This event probably is resulted from the energy availability of the fat sources used in this trial and the fact that birds consumed the amount of feed to meet their nutritional energy demands. Our results are consistent with those of Panja *et al.* (1995). In both studies, the ME intakes of the birds (layers and broilers) were constant irrespective from the available energy content of palm fatty acid-diets.

Feed conversion efficiency did not differ between experimental groups during the period of 27 to 32 wk of age (Table 2). Increase in dietary substitution of PFAP for SO, particularly energizer A, increased feed conversion ratio (FCR) during 32–37 wk of age. The birds on energizer B-

Table 3. Effects of dietary replacement of soybean oil with two commercial fat sources on egg weight and egg albumen and yolk quality at 32 and 37 wks of age

Fat source[1] and replacement level (%)		Soy-oil	Egg production (g)		Haugh unit		Yolk index	
			27 to 32 wk	32 to 37 wk	27 to 32 wk	32 to 37 wk	27 to 32 wk	32 to 37 wk
Control		100	58.19	57.40	83.40	84.58	0.429	0.442
A	25	75	58.02	58.14	85.12	85.02	0.446	0.456
	50	50	59.87	59.76	87.10	87.35	0.458	0.455
	75	25	58.50	58.80	86.37	83.78	0.469	0.461
	100	0	58.22	58.79	84.73	81.81	0.464	0.464
B	25	75	59.76	59.90	88.14	84.39	0.438	0.447
	50	50	59.25	59.07	86.11	81.74	0.450	0.454
	75	25	59.83	59.29	86.52	84.86	0.449	0.464
	100	0	59.17	59.14	85.97	81.85	0.458	0.459
			Probability					
Treatment			NS	NS	NS	NS	*	*
Contrasts								
Control vs. others			NS	NS	*	NS	**	**
Replacement level			NS	NS	NS	NS	**	**
A vs. B			0.0541	NS	NS	NS	NS	NS
100% of three sources			NS	NS	NS	NS	*	0.0714
Pooled SEM			0.6117	0.8721	1.2865	1.4089	0.0075	0.0046

[1]Fat sources were used in place of soybean oil in control diet, were fatty acid powders of palm oil (energizer A as granulated fat powder and energizer B as Ca salt of FA).NS: not significant; *$P<0.05$; **$P<0.01$.

Table 4. Effects of dietary replacement of soybean oil with two commercial palm fat sources on yolk colour index and egg shell quality at 32 and 37 wks of age

Fat source[1] and replacement level (%)	Soy oil	Yolk colour score		Shell strength (kg/cm^2)		Shell thickness (mm)	
		27 to 32 wk	32 to 37 wk	27 to 32 wk	32 to 37 wk	27 to 32 wk	32 to 37 wk
Control	100	8.90	8.95	3.20	3.33	0.440	0.426
A 25	75	8.75	9.70	3.28	3.39	0.432	0.427
50	50	8.95	9.25	3.45	3.47	0.430	0.429
75	25	9.30	10.15	3.26	3.50	0.439	0.436
100	0	9.75	9.95	3.37	3.63	0.438	0.427
B 25	75	9.30	9.90	3.26	3.59	0.446	0.433
50	50	9.35	10.10	3.44	3.82	0.449	0.439
75	25	9.05	10.00	3.51	3.70	0.448	0.435
100	0	8.85	10.10	3.33	3.69	0.448	0.435
		Probability					
Treatment		NS	NS	NS	*	*	*
Contrasts							
Control vs. others		NS	*	NS	*	NS	NS
Replacement level		NS	NS	NS	NS	NS	NS
A vs. B		NS	NS	NS	**	***	**
100% of three sources		NS	NS	NS	**	NS	NS
Pooled SEM		0.3066	0.3532	0.0879	0.0917	0.0042	0.0029

[1]Fat sources were used in place of soybean oil in control diet, were fatty acid powders of palm oil (energizer A as granulated fat powder and energizer B as Ca salt of FA).NS: not significant; *P<0.05; **P<0.01; ***P<0.001. A, B are energizers.

diets had a better (P<0.01) FCR than A-fed groups.

Egg weight was similar among the experimental groups during 27 to 32 wk of age, although the birds on energizer B-diets tended (P=0.0541) to lay heavier eggs than those on energizer A-diets (Table 3). On the other hand, dietary inclusion of PFAP in substitution for SO tended (P=0.0582) to increase egg weight during the second 35-d experimental period. This effect may be, in part, due to the synergistic effects of USFA of soy oil on SFA contained in PFAP, whereby USFA promote micelle formation into the small intestine and help SFA to absorb more efficiently from the absorptive epithelial cells (Scott *et al.* 1982, NRC 1994). When SFA absorbed, provide more energy than that of unsaturated ones because of their chemically more reduced structure (Voet and Voet 1995). Haugh scores had no significant differences during both periods of 27 to 32 and 32 to 37 wk of age (Table 3). Of course, dietary inclusion of PFAP improved (P<0.05) albumen quality during the first trial period. The exact mechanism(s) involved, however, remains to be elucidated.

Yolk index (Table 3) as a measurement of yolk standing-up quality was higher (P<0.01) in groups fed on palm fatty acid-containing diets than that of control birds. There was no considerable difference between 2 commercial PFAP in the light of yolk index. In contrast, increase in dietary replacement level of both PFAP caused the significant (P<0.01) increases in yolk quality index. Presumably, the higher yolk index in eggs of PFAP-groups can be attributed to SFA profile of these fat sources. Saturated fatty acids are expected to increase yolk standing-up.

The data on yolk colour score and egg external quality measurements (shell thickness and eggshell breaking strength) are presented in Table 4. Yolk colour index improved (P<0.05) by dietary PFAP inclusion but only during the second 35-d period. The oxycarotenoids contained in the diet (some of them derived from PO) are fat soluble; therefore, their absorption from the intestine is likely to be improved when fed in parallel with the dietary lipids (Hamilton and Parkhurst 1990, Grobas *et al.* 2001).

Both shell thickness and eggshell breaking strength improved (P<0.01) by inclusion of energizer B into the diet; however, the shells of eggs produced by hens on A-diets did not differ from those laid by control hens in thickness or strength. It appears that the Ca contained in energizer B is responsible for major part of eggshell quality improvements. In addition, a part of egg production and egg mass responses to energizer B is probably related to the Ca contained in this PFAP source. As pointed previously, the most of the Ca soaps of unsaturated FFA are absorbed by the birds, and their energy and mineral contents retained into the body (Atteh and Leeson 1984, 1985).

Yolk cholesterol (Table 5) was significantly (P<0.05) higher for hens fed on palm fatty acid-diets than for hens on control diet. Almost the linear pattern was observed in egg yolk cholesterol with increasing replacement level of palm fatty acids. On the other hand, triglycerides content of egg yolk did not differ between the experimental groups. This finding was not unexpected because reports showed that there

Table 5. Effects of dietary replacement of soybean oil with two commercial palm fat sources on egg yolk indexes at 37 wks of age

Fat source[1] and replacement level (%)		Soy oil	Yolk cholesterol (mg/g of yolk)	Yolk TG (mg/g of yolk)	Yolk MDA (nmol/g of yolk)
Control		100	9.65	253	337
A	25	75	10.21	238	272
	50	50	10.74	249	199
	75	25	11.73	273	151
	100	0	12.47	266	167
B	25	75	9.43	261	341
	50	50	9.92	242	241
	75	25	10.82	233	184
	100	0	11.63	257	171
Probability					
Treatment			**	NS	**
Contrasts					
Control vs. others			*	NS	*
Replacement level			***	NS	***
A vs. B			NS	NS	NS
100% of three sources			**	NS	*
Pooled SEM			0.80	12.73	38.50

[1]Fat sources were used in place of soybean oil in control diet, were fatty acid powders of palm oil (energizer A and energizer B).NS: not significant; *P<0.05; **P<0.01; ***P<0.001. A and B are energizers.

is a close relationship between intake of SFA and plasma concentrations of cholesterol (Kromhout 1992). The fact that the highest levels of dietary inclusion of PFAP were associated with the higher cholesterol concentrations in the egg yolks indicates that dietary utilization of PFAP high in SFA content, should be confined to 50% in replace of SO.

The finding of significant importance was the reduction (P<0.05) of yolk MDA index by dietary inclusion of PFAP (Table 5). The decrease in MDA content of egg yolk from hens fed on palm fatty acids-containing diets may be attributed mainly to its SFA profile. A fact which confirms this explanation is the absence of significant difference between yolk MDA of energizer A- and energizer B-hens. Further investigations are needed, however, to demonstrate this hypothesis.

On the basis of these observations, we conclude that palm oil fatty acids can potentially replace a part (about 75%) of conventional dietary fat sources; however, the original dietary fat source should be of plant origin or a USFA-rich source to benefit from synergistic effects occurred between unsaturated and saturated fats. Because the Energizer B has a more unsaturated free fatty acid profile and also contained in Ca, this source appears to be superior for use in laying hen's diet compared with Energizer A powder.

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