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Dietary distilled fatty acids and antioxidants improve nutrient use and performance of Japanese male quails

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Distilled fatty acids (DFA) are by-products of edible oil refining and may be used in animal nutrition. This study was carried out to evaluate the effect of DFA and vitamin E (VE) and/or selenium (Se) on digestibility, performance, and meat quality of male Japanese quails. Seven hundred and twenty male quails were used in a completely randomized design in a 3×2×2 factorial arrangement, totaling 12 treatments and four replicates with 15 birds each. Treatments consisted of three DFA levels (0%, 3%, and 6%), two VE levels (0 and 50 ppm) and two Se levels (0 and 0.2 ppm). Six birds per treatment were slaughtered for carcass measurements. The inclusion of VE in diets with 3% DFA improved the dry matter use, while inclusion of VE and Se without DFA increased crude protein and ether extract use. Inclusion of DFA or Se resulted in the best feed:gain ratio. Quails fed diets without DFA, VE, and Se showed a higher feed intake compared with the other treatments. In turn, inclusion of Se and VE improved ($P<0.05$) contents of moisture and crude protein in the meat. Tenderness was increased ($P<0.05$) due to the VE inclusion in the diets. It was concluded that VE and organic Se may be included in diets containing up to 6% of DFA for male Japanese quails in view of the resulting improvement in nutrient use, productive performance and meat quality.

KEY WORDS: free radical scavengers / oils and fats industry / quail nutrition

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Distilled fatty acids (DFA) are by-products of edible oil refining and usually contain a high proportion of free fatty acids and a small proportion of neutral oils. They may be used in poultry nutrition; however, fats and/or oils and especially free fatty acids are very susceptible to oxidation. This may lead to the development of rancidity and color or texture changes [Taulescu *et al*. 2011], having a negative effect on food and feed quality and thus on human and animal health.

Selenium (Se) belongs to the group of oligoelements found in glutathione peroxidase, an essential enzyme for tissue protection against free radicals and oxidation. This mineral improves the function of the immune system and plays an important role in the animal's growth, as it may affect the activity of thyroid hormones [Tinggi 2008]. The additions of vitamin E (VE) and Se are important when feed is supplemented with fatty acids. VE and Se are beneficial in preventing the detrimental effects of diets enriched with polyunsaturated fatty acids (PUFA), because they are important components of the antioxidant system, reducing the lipid peroxidation [Taleuscu *et al*. 2011]. Chitra *et al*. [2016] reported that 0.3 ppm Se + 150 ppm VE improved growth rate and feed conversion rate in Japanese quail broilers. However, several authors have reported no effect of Se and/or VE supplementation in diets for broilers on their body weight [Skrivan *et al*. 2008] and feed intake [Malayoglu *et al*. 2009].

Albuquerque *et al*. [2017] found that the addition of Se and VE in broilers' diets did not improve the carcass and organ weights. In turn, Mousa *et al*. [2017] used organic Se and VE (5g/kd diet) for chicks and noted an improvement in carcass yield and meat chemical composition. The inclusion of antioxidants in animal diets may improve meat quality. Colour and lipid stability are important factors for acceptability and quality of meat. VE and Se may improve meat quality by preventing lipid oxidation [Skirivan *et al*. 2008].

The aim of this study was to evaluate a potential for replacing 3% dietary sunflower oil with 0%, 3%, and 6% DFA, as well as the effect of VE and/or organic Se on digestibility, productive performance, carcass traits and meat quality of male Japanese quails during the period of 21-49 days of age.

Material and methods

This work was approved by the scientific and ethical committee of the Department of Animal and Poultry Production, the Faculty of Agriculture, Damanhour University (Egypt). Seven hundred and twenty male Japanese quails with 96.1±1.26 g average initial weight were used in a completely randomized design in a 3×2×2 factorial arrangement, totaling 12 treatments with four replicates of 15 individuals each. Males were used in this study because of their high growth rate, the expectation that they represented a better experimental model than females and greater homogeneity within treatment variations. Treatments consisted of three DFA levels (0, 3, and 6%), two VE levels (0 and 50 ppm; α-tocopherol acetate, Agrifax Co., David Knight, UK), and two organic Se levels (0 and 0.2 ppm; Sel-Plex®, Alltech Inc., Kentucky, United States).

Table 1. Composition of experimental diets

	Distilled fatty acid (%)		
Ingredients (kg)	0.0	3.0	6.0
Yellow corn	45.00	44.00	42.50
Soybean meal	47.20	47.70	48.10
Distilled fatty acid	0.00	3.00	6.00
Wheat bran	1.40	0.38	0.00
Sunflower oil	3.00	1.50	0.00
Limestone	0.45	0.42	0.40
Dicalcium phosphate	2.05	2.10	2.10
Min-Vit. premix[1]	0.30	0.30	0.30
Common salt	0.30	0.30	0.30
DL-methionine	0.30	0.30	0.30
Total	100	100	100
Calculated composition			
Metabolisable energy (kcal/kg)	2879	2882	2874
Crude protein (%)	24.99	24.96	24.95
Total methionine (%)	0.67	0.67	0.69
Total sulfur amino acids (%)	1.06	1.06	1.06
Total lysine (%)	1.40	1.40	1.40
Calcium (%)	1.06	1.07	1.01
Available phosphorus (%)	0.50	0.50	0.50
Selenium (mg/kg)	0.17	0.16	0.16
Vitamin E (mg/kg)	21.00	20.70	20.30

[1]Provided per kg of diet: vitamin A 12000 IU; vitamin E 10 IU; vitamin K_3 3 mg; vitamin D_3 2200 IU; riboflavin 10 mg; calcium pantothenate 10 mg; niacin 20 mg; choline chloride 500 mg; vitamin B_{12} 10 µg; vitamin B_6 1.5 mg; thiamine 2.2 mg; folic acid 1 mg; D-biotin 50 µg, Mn 55 mg; Zn 50 mg; Fe 30 mg; Cu 10 mg; Se 0.1 mg, and ethoxyquin 3 mg.

Table 2. Fatty acid composition (% of total fatty acids) of distilled fatty acid and sunflower oil

Fatty acid	Carbon atom	Distilled fatty acid	Sunflower oil
Capric	C10:0	-	0.349
Lauric	C12:0	-	0.899
Myristic	C14:0	0.225	1.099
Palmitic	C16:0	14.597	18.914
Stearic	C18:0	5.929	7.303
Arachidic	C20:0	13.025	6.517
Behenic	C22:0	0.599	0.499
Palmitoleic	C16:1	0.262	0.874
Oleic	C18:1	24.193	26.217
Linoleic	C18:2	41.170	37.328
SFA[1]		34.375	35.580
UFA[1]		65.625	64.420
SFA/UFA[1]		0.524	0.552
MUFA[1]		24.455	27.090
PUFA[1]		41.170	37.328
PUFA/MUFA[1]		1.684	1.378

[1]SFA saturated fatty acid; UFA unsaturated fatty acid; MUFA monounsaturated fatty acid; PUFA polyunsaturated fatty acid.

The compositions of the experimental diets, fatty acids of DFA and sunflower oil are found in Tables 1 and 2. The experimental diets had comparable nutritive values.

Quails were housed in cages (50×60×50 cm) from 21 to 49 days of age and received water and mash feed *ad libitum*.The lLight program was provided with a 24 h light/day cycle. Birds were weighed at 21 and 49 days of age and body weight gain (BWG) was calculated. Feed intake (FI) was recorded for each replicate and thereby the feed: gain ratio (FGR) was determined.

An assay of apparent digestibility was carried out from 49 to 54 days of age using the method of total gut collection of excreta. Trays lined with plastic canvas were fit under the cages for excreta collection. Excreta were collected twice a day, placed in labelled plastic bags and stored in a freezer at -18°C until the end of the collection period. The samples were thawed, homogenized per experimental unit, weighed and submitted to the laboratory analysis. In the laboratory samples were pre-dried in a ventilated oven at 65°C for 72 h. Dried samples were then weighed, ground and analysed for dry matter (DM), crude protein (CP) and ether extract (EE) (AOAC, 2005), while the digestibility coefficient was calculated as follows:

$$\text{DCDM (\%)} = \frac{\text{DM}_{\text{ingested(g)}} - \text{DM}_{\text{excreted(g)}}}{\text{DM}_{\text{ingested(g)}}} \text{ x } 100$$

where: DCDM stands for digestibility coefficient of dry matter, DM for dry matter.

At 49 days of age, six male quails were randomly selected per treatment for slaughter, totaling 72 birds. Their body weight was determined 12 h after feed withdrawal and birds were slaughtered in an experimental processing plant. Weights of the liver, heart, gizzard, spleen, and pancreas were recorded. The relative weight of the carcass and internal organs was expressed as the percentage of slaughter weight. Chemical composition and physical characteristics of the meat were determinedd using a sample of 50% breast meat + 50% thigh meat. Contents of moisture, CP and EE of meat, feeds and excreta were determined according to AOAC (2005) and expressed on a dry matter basis. Meat tenderness and water holding capacity (WHC), pH and colour were analysed as described by Aggoor *et al.* [2000].

Statistical analysis was conducted using the SISVAR® software [Ferreira 2011], with the differences between means of the main factors or the factorial arrangement examined by Tukey'a test.

Results and discussion

The interaction of DFA × VE × Se levels was significant for the digestibility coefficient (DC) of DM, CP and EE. The inclusion of VE in diets with 3% DFA improved DCDM, while dietary inclusion of VE and Se without DFA increased DCCP and inclusion of VE and Se increased DCEE in diets without DFA (Tab. 3). In general, addition of VE and/or Se in diets containing 6% DFA did not improve the

Table 3. Digestibility coefficient (%) of nutrients and productive performance in Japanese male quails fed diets containing differing levels of distilled fatty acid (DFA) and supplemented with vitamin E (VE) and/or selenium (Se)

Treatment	Dry matter	Crude protein	Ether extract	Body weigh gain (g)	Feed intake (g)	Feed: gain ratio
			Effect of distilled fatty acid levels (%)			
0	82.00[a]	79.54[a]	83.55[a]	77.52	308[a]	3.99[a]
3	80.62[b]	76.50[b]	83.50[a]	80.95	301[b]	3.67[b]
6	79.46[c]	76.57[b]	81.84[b]	82.65	300[b]	3.72[b]
			Effect of Vitamin E levels (ppm)			
0	81.21	77.58	82.17	80.50	304	3.81
50	80.03	77.33	83.67	80.23	302	3.79
			Effect of Selenium levels (ppm)			
0	80.85	77.75	81.67	78.95	310[a]	3.94[a]
0.2	80.40	77.17	84.17	81.79	296[b]	3.65[b]
			Effects of interaction distilled fatty acid × Vitamin E × Selenium levels			
0×0×0	82.60[b]	79.00[b]	81.50[c]	76.88	331[a]	4.31
3×0×0	80.50[c]	78.00[b]	83.00[c]	76.16	306[c]	3.95
6×0×0	81.02[bc]	77.87[b]	79.25[d]	78.67	300[c]	3.93
0×0.2×0	80.70[c]	78.00[b]	81.00[c]	79.87	286[b]	3.60
3×0.2×0	85.00[a]	77.00[bc]	85.00[b]	87.28	296[c]	3.42
6×0.2×0	78.00[c]	76.00[c]	84.00[b]	84.16	303[c]	3.60
0×0×50	79.00[c]	81.00[a]	84.00[b]	77.99	310[bc]	3.99
3×0×50	80.30[bc]	76.00[bc]	82.00[c]	86.21	316[b]	3.68
6×0×50	82.20[b]	75.00[c]	81.00[c]	77.79	294[c]	3.78
0×0.2×50	82.70[b]	80.00[a]	87.00[a]	75.31	304[c]	4.05
3×0.2×50	78.00[c]	75.00[c]	84.00[b]	80.93	291[d]	3.64
6×0.2×50	78.00[c]	77.00[b]	84.00[b]	83.16	296[c]	3.58
SEM	0.28	0.29	0.17	1.55	4,15	0.14
			p-value			
DFA × VE × SE	0.001	0.030	0.008	0.184	0.001	0.297
DFA × VE	0.001	0.001	0.001	0.696	0.419	0.754
DFA × SE	0.001	0.147	0.013	0.492	0.001	0.948
DFA	0.001	0.001	0.001	0.072	0.014	0.008
VE	0.001	0.448	0.001	0.880	0.493	0.835
SE	0.176	0.084	0.001	0.122	0.001	0.001

SEM – standard error of the mean.
[ab…]Within columns means bearing different superscripts differ significantly at P≤0.05.

nutrient digestion and absorption, in contrast to the diets without or with 3% DFA. VE and Se act as antioxidants that may prevent damage in the brush border enzyme activity and protect pancreatic tissues against oxidative damage, thus resulting in the best functioning of these organs. According to Lenzen *et al.* [1996], the ability of pancreatic β-cells to counteract oxidative stress is limited, because the expression level of antioxidant enzymes: superoxide dismutase, glutathione peroxidase and mainly catalase is substantially lower than in most other tissues. Insulin and glucagon are important hormones associated with the energetic metabolism.

Since the dietary supplements improved DM, CP and EE digestibility, BWG did not change, while FI was reduced due the VE and Se inclusions in diets containing DFA. These results are in an agreement with those presented by Mobaraki and

Shahryar [2015], who reported that 160 mg/kg VE + 0.4 mg/kg Se reduced FI in Japanese quails. On the other hand, Marques *et al*. [2011] and Chitra *et al*. [2016] observed no differences in FI of quails supplemented with dietary VE.

BWG was not affected (P<0.05) by the treatments; however, dietary inclusion of DFA or Se resulted in best FGR values. FI was influenced (P>0.05) by the interaction of DFA×VE×Se; quails fed diets without DFA, VE and Se showed a FI up to 13.47% higher compared with the other treatments (Tab. 3). The best values of FCR due the DFA supplementation could be attributed to the reduced rate of passage of digesta in the gastrointestinal tract, which promotes better absorption of all nutrients present in the diet. Selenium inclusion improved FCR, probably due its presence in the structure of GSH-Px [Hamam and Abou-Zeina 2007], an important antioxidant enzyme, as well as the improvement of the bird's metabolism and nutrient use. Our results are in agreement with the findings of Zia *et al*. [2016], while they differ from those obtained by Cruz and Fernandez [2011].

Table 4. Slaughter weight (SW) and carcass traits of Japanese male quails fed diets containing differing levels of distilled fatty acid (DFA) and supplemented with vitamin E (VE) and/or selenium (Se)

Treatments	SW	Relative weight (%)					
		Carcass	Heart	Gizzard	Liver	Pancreas	Spleen
		Effects of distilled fatty acid level (%)					
0	151.46	74.90	1.29	2.58	2.80^{a}	0.26	0.052^{b}
3	151.15	75.13	1.32	2.52	2.53^{b}	0.24	0.045^{b}
6	152.67	75.72	1.34	2.65	2.58^{b}	0.24	0.078^{a}
		Effects of Vitamin E level (ppm)					
0	152.13	75.03	1.35	2.64	2.58	0.24	0.062
50	152.05	75.47	1.28	2.54	2.70	0.25	0.054
		Effects of Selenium level (ppm)					
0	151.11	75.70	1.32	2.57	2.64	0.24	0.055
0.2	153.07	74.80	1.31	2.59	2.65	0.26	0.062
		Effects of interaction distilled fatty acid × Selenium levels					
0×0	147.42	76.84	1.31	2.47	2.61^{b}	0.24	0.045
3×0	152.67	74.79	1.30	2.51	2.63^{b}	0.24	0.051
6×0	153.25	74.93	1.35	2.74	2.67^{b}	0.23	0.072
0×0.2	157.50	74.35	1.27	2.70	3.00^{a}	0.28	0.060
3×0.2	149.63	73.79	1.34	2.53	2.43^{b}	0.23	0.040
6×0.2	152.08	75.38	1.32	2.57	2.51^{b}	0.25	0.084
SEM	4.21	0.56	0.04	0.09	0.19	0.08	0.005
				p value			
DFA × VE × SE	0.267	0.917	0.264	0.649	0.571	0.114	0.091
DFA × VE	0.511	0.143	0.987	0.753	0.903	0.666	0.055
DFA × SE	0.066	0.333	0.117	0.833	0.010	0.759	0.151
DFA	0.858	0.577	0.685	0.968	0.087	0.452	0.001
VE	0.977	0.498	0.912	0.349	0.402	0.648	0.174
SE	0.424	0.178	0.659	0.488	0.549	0.493	0.280

SEM – standard error of the mean.

[ab]Within column means bearing different superscripts differ significantly at P≤0.05.

Table 5. Meat characteristics of Japanese male quails fed diets containing differing levels of distilled fatty acid (DFA) and supplemented with vitamin E (VE) and/or selenium (SE)

Treatments	Moisture (%)	Protein (%)[1]	Fat (%)[1]	Water holding capacity	pH	Tenderness	Colour
			Effects of distilled fatty acid level (%)				
0	67.18	70.29[b]	21.91	5.71	6.61	3.02	0.298
3	67.50	71.02[a]	21.24	5.66	6.58	3.01	0.298
6	65.92	69.81[c]	21.37	5.65	6.60	3.02	0.302
			Effects of Vitamin E level (ppm)				
0	67.17	70.12	23.01[a]	5.64	6.58	2.96[b]	0.302
50	66.50	70.59	20.00[b]	5.71	6.62	3.07[a]	0.290
			Effects of Selenium level (ppm)				
0	66.50	71.20	20.82[b]	5.64	6.58	3.00	0.301
0.2	67.17	69.52	22.19[a]	5.71	6.61	3.03	0.292
			Effects of interaction distilled fatty acid × Vitamin E × Selenium levels				
0×0×0	66.50[c]	72.65[a]	22.03	5.70	6.63	3.03	0.292
3×0×0	65.00[c]	72.90[a]	20.53	5.52	6.60	2.89	0.300
6×0×0	70.25[a]	71.95[b]	21.36	5.55	6.51	2.91	0.322
0×0×0.2	68.00[ab]	65.59[d]	25.28	5.67	6.61	2.93	0.290
3×0×0.2	69.00[a]	67.38[c]	23.06	5.73	6.60	2.98	0.312
6×0×0.2	63.00[d]	70.53[b]	25.77	5.69	6.51	3.06	0.297
0×50×0	67.00[bc]	68.54[c]	20.63	5.74	6.62	3.06	0.290
3×50×0	67.00[bc]	70.59[b]	20.20	5.68	6.62	3.08	0.302
6×50×0	62.00[d]	70.81[b]	20.16	5.68	6.66	3.05	0.300
0×50×0.2	67.00[bc]	73.11[a]	19.69	5.74	6.60	3.06	0.282
3×50×0.2	69.00[a]	69.85[c]	21.15	5.72	6.58	3.09	0.280
6×50×0.2	67.00[bc]	70.64[b]	18.19	5.68	6.62	3.08	0.287
SEM	0.45	0.42	0.33	0.05	0.06	0.06	0.011
				p value			
DFA×VE×SE	0.001	0.001	0.053	0.429	0.444	0.416	0.460
DFA × VE	0.022	0.001	0.004	0.945	0.127	0.668	0.823
DFA × SE	0.004	0.001	0.804	0.227	0.339	0.277	0.737
DFA	0.045	0.001	0.338	0.313	0.853	0.941	0.375
VE	0.215	0.001	0.001	0.069	0.688	0.006	0.072
SE	0.215	0.001	0.001	0.066	0.206	0.373	0.172

[1]On a dry matter basis.
SEM – standard error of the mean.
[ab…]Within columns means bearing different superscripts differ significantly at P≤0.05.

Slaughter weight and carcass traits were not affected (P>0.05) by the treatments, except for liver and spleen relative weight (P<0.05). Spleen had its relative weight improved by diets with 6% DFA. The interaction of DFA×Se was significant for the liver, as its relative weight was greater when Se was added to the diets with no DFA (Tab. 4). Inclusion of Se and VE improved (P<0.05) contents of moisture and CP in the meat (Tab. 5). When added separately, VE reduced (P<0.05) and Se increased (P<0.05) fat content in the meat. Tenderness was increased (P<0.05) due to VE inclusion in the diets. The treatments affected only relative weight of the spleen and liver. These results suggested that the experimental diets had no adverse effect on bird metabolism and that Japanese quail males could tolerate up to 6% DFA at 21-49 days of age without adverse effects on their carcass yield and relative weight of organs. Pancreatic enzyme secretion is strongly stimulated by dietary lipids. However, studies on humans and birds showed that, within a certain range, the energy content is more important for pancreatic enzyme response than the concentration of an individual nutrient [Keller and Layer 2005, Polycarpo *et al*. 2014].

Inclusion of DFA at 3% and 6% reduced the liver relative weight and this effect was also mentioned in an earlier study by Bray and Krauss [2014]. The authors explained that the consumption of more dietary PUFA than saturated fatty acids (SFA) resulted in lower liver and visceral fat contents in humans. Ferramosca *et al.* [2014] reported that a diet enriched in PUFA inhibits hepatic fatty acid synthesis more strongly than a diet enriched in monounsaturated fatty acids or SFA. Inclusion of Se increased liver weight in diets with no DFA, because it stimulates hepatic lipogenesis and gluconeogenesis, as well as increases the liver protein concentration [Zhao *et al.* 2016].

Spleen weight was increased due to the 6% DFA addition in the diets. Dietary fat level may cause splenomegaly via sinusoidal dilatation and extracellular deposits such as lipid and hemosiderin [Altunkaynak et al. 2007], or because it increases the white pulp and germinal center hyperplasia in the spleen [Silva *et al*. 2012].Contents of moisture and CP increased in the meat, while fat content was reduced. Different results were found by researchers who worked with Se and VE supplementation in diets for pigs [Svedaite *et al*. 2009], geese [Lukaszewicz *et al.* 2016] and Nelore cattle [Carmo *et al.* 2017], where no differences in meat DM, fat and protein contents were observed. However, these results differ from those of Lukaszewicz *et al*. [2016]. Addition of Se in the diets resulted in a higher fat content in the meat, probably because Se mediates several insulin-like actions both *in vivo* and *in vitro*. These insulin-like actions include fatty acid synthesis [Coulibaly *et al.* 2011]. One of the effects of insulin on muscle is to increase the uptake of triglycerides from the blood, stimulating fatty acid and triacylglycerol synthesis, while decreasing the rate of fatty acid oxidation [Dimitriadis *et al.* 2011], which in turn may increase fat content in the meat.

Tenderness was increased due to VE inclusion in the diets. VE can improve meat quality, because it prevents damage to cells throughout the body by maintaining cell membrane integrity [Moravej *et al*. 2012] and reducing cellular water losses. Oxidation manifests as a conversion of the red muscle pigment myoglobin to brown metmyoglobin [Castillo *et al*. 2013], and the use of antioxidants such as VE and Se may delay this conversion. However, colour was not altered in this study. Water-holding capacity is important, since it influences meat tenderness, taste and yield. Following the glycolysis process, the amount of lactic acid from meat increases, the pH decreases, whereas WHC decreases [Ianitchi *et al.* 2008]. In this study no effect was observed on WHC or pH in the meat; however, tenderness was greater at the VE addition. Sethy *et al*. [2014] also reported no effect on pH and WHC and an improvement in tenderness of goat meat using VE alone and at a simultaneous addition of VE and Se.

It was concluded that VE and organic Se may be included in diets for male Japanese quails containing up to 6% of DFA in view of the improvement in nutrient use, productive performance and meat quality.

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