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The influence of feeding laying hens N-3 and N-6 fatty acids sources on blood antioxidants status: A comparative study

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Abstract: The objective of this study is to determine the effects of dietary supplementation with different fat sources on blood oxidative status parameters. Seventy five (Hisex) birds, 20 week old, were obtained from Animal Production Research Center (kuku), divided into three groups (n=25) for each one. The control group (A) was under control diet based on corn, maintains the (NRC, 1994) requirements for laying hens, the experimental groups were supplemented by 10% flax seeds (B) and 10% sunflower seeds (C), added to the diet. The trial continued for eight weeks, blood samples were collected once per month (week 4 and 8), in EDTA coated vials, immediately placed into iced-container, centrifuged at 3000rpm/20 min. Samples were separated in aliquot and stored at -20°C and -80°C until analysis.

Plasma analysis for enzymatic and non-enzymatic antioxidants, revealed no significant different level in uric acid, malondialdehyde (MDA) and vitamin C, between the control group and the treated groups, while catalase (CAT), superoxide dismutase (SOD), and vitamin A, levels were significantly enhanced by the addition of 10% flax seeds and 10% sunflower seeds. Vitamin E concentration was significantly elevated in group (C), compared to the control group (A) and group (B).

Key words: Oxidative status, Sunflower seeds, flax seeds, Layers, SOD, CAT, vitamin E.

Introduction

Oxidative stress is defined as an imbalance between production of free radicals and reactive metabolites, so-called oxidants or reactive oxygen species and their elimination by protective mechanisms, referred to as antioxidants. This imbalance leads to damage of important biomolecules and cells, with potential impact on the whole organism [1]. The harmful effects of ROS are balanced by the action of antioxidants, some of which are enzymes present in the body [2]. Despite the presence of the cell antioxidant defense system to counteract ROS oxidative damage, this latter accumulates during the life cycle and has been implicated in diseases, aging and age dependent diseases such as cardiovascular disease, cancer, neurodegenerative disorders and other chronic conditions [3].

The term "antioxidant" refers to any molecule capable of stabilizing or deactivating free radicals before they attack cells. Humans have evolved highly complex antioxidant systems (enzymatic and non-enzymatic), which work synergistically, and in combination with each other to protect body cells and organ systems against free radical damage. The antioxidants can be endogenous or obtained exogenously for example as a part of a diet or as dietary supplements. Some dietary compounds that do not neutralize free radicals, but enhance endogenous activity may also be classified as antioxidants [4].

Today's poultry producers are confronted with numerous challenges to prevent diseases and maintain health without the use of sub-therapeutic antibiotics. As food safety and animal welfare concern continue to increase, researchers will continue to seek better alternatives to current methods applied to molting laying hens [5] and [6].

Natural dietary antioxidants are currently receiving considerable attention in animal nutrition fields due to their association with feed high-quality characteristics and their contribution to the protection against oxidant stress.

Peroxides are reactive oxygen species (ROS) that cause oxidative damage which is together with excessive oxidative stress two consequences of insufficient antioxidant potential in animals [7].

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Under normal circumstances, the animal could remove excessive ROS using a non-enzymatic antioxidant system and a series of antioxidant enzymes such as catalase (CAT) and superoxide dismutase (SOD) [7] and [8].

A high antioxidative status has been regarded as one of the major factors positively affecting the production performance in the intensive poultry industry [5] and [9].

The ω-3 and ω-6 PUFAs play an important role in health and treatment of diseases. They act as antibacterial agents, [10]; [11] and [12], anti-inflammatory agents [13] and [14], or antioxidants [15]. They are also effective in the treatment of cardiovascular diseases [16], and cancer cell proliferation [17] and [18]. Such properties are indicative of the potentiality for PUFAs as nutraceuticals and pharmaceuticals. The (ω-3/ω-6) ratio can significantly influence the body metabolic function [19]; where an increased intake of ω-3 PUFAs leads to the replacement of ω-6 PUFAs in cells and tissues resulting in a reduction of the overall ω-3/ω-6 ratio. Also a reduction of arachidonic acid [20]; derived metabolites has been observed. Dietary intake of ω-3 fatty acids may prevent the development of the previously cited disease.

The positive effect of linseed oil on blood parameters, represented by a significant increase in the value of the total antioxidative potential and a decrease in the amount of lipid peroxidation products (hydrogen peroxide and malondialdehyde), makes the application of linseed oil as a source of fat in feeding turkey hens considered as advisable [21].

Moreover, the inclusion of n-3 PUFA in diets at moderate levels increase the n-3 PUFA content in meat, in Japanese quail [22]. The concentration of vitamin E inplasma, increases remarkably when they are fed sunflower oil containing diet [23].

An important source of PUFA (N-3 Fatty acids) is flaxseed obtained from *Linum usitatissimum* plants (Linaceae family), cropped mainly in Argentina, Brazil, Canada, China, India and Turkey [24]. Flaxseed is a flat, oval-shaped seed [25]; whose oil contains 53% alpha-linolenic acid (ALA), an essential ω-3 fatty acid. Flaxseed is also a good source of dietary fiber (20-25%) [26]; and lignans (500 µg/g), which are plant steroids analogous to mammalian estrogen [27].

While PUFA (N-6 Fatty acids) are mostly found in sunflower seeds (*Helianthus annuus* L) which is a coarse, stout and erect annual plant 1-3 meters high. It produces greyish green or black seeds encased in tear-dropped shaped grey or black shells that often times feature black and white strips. Seeds encased in plant contain monoterpenes (α-pinene, Sabinene) [28] and [29], diterpenes (Helikauranoside) [30]; oleic acid, triacyl glycerol, alkaloids, cyanogenic glycosides, saponins, cardiac glycosides, tannins, fixed oils, flavonoids [31]; sesquiterpenes lactones [30]; alkaloids [32]. Flowers contain quercimeritrin, anthocyanin, abundant amount of cholin and betain, triterpene [33]; saponins [34]. Seeds contain 45 to 48 percent fixed oil, tannins [35]; polyphenols [36].

Objectives:

- The aim of this study is to evaluate the effect of omega-6 and Omega-3 fatty acids from plant sources, on the blood oxidative status of laying hens.
- The discovered plant source can be added to laying hens diets to improve their oxidative status and health and in subsequence to improve the production parameters, quantitatively and qualitatively.

Materials and methods

The experiment was held in the Veterinary Research Institute (VRI), from January to march 2014. The duration of the experiment was 8 weeks.
Four, full wire cages were prepared, each cage measures 2X 1.5X 1 meter and can contain15 birds. The cages were placed at an open poultry house.
Seventy five laying hens (Hisex) breed, 20 week old, obtained from animal production research center (kuku), were evaluated in this study. The birds were divided into three groups, 25 birds per group.

Experimental Diets: The diets were formulated to meet the requirements of egg production according to the directions of the national research council [37]. Two experimental formulae were prepared by insertion of 10% flax seeds and 10% sunflower seeds.

The supplementary sources were subjected to proximate analysis, to determine their content of protein, fat, fiber, nitrogen free extract (N.F.E) and energy **(Table.1).**

Table 1. Proximate analysis of seeds

	D.M (Dry matter)%	Moisture%	Protein %	Fat %	Fiber%	Ash%	N.F.E%	Energy%
Flaxseeds Seeds	96.55	3.45	27.84	35.69	10.78	4.67	17.57	3932.0
Sunflower seeds	98	2	26.00	36.49	11	6	18.5	3054.07

❖ **Management:**

Each group received its diet from day one **(Tables.2 and 3).** Drinking system contained two tanks for each cage, the tanks were cleaned, and the water was changed twice daily. Birds received 24 hour light/day throughout the experiment.

Three ml of blood was collected from twenty bird of each group, the blood was taken using a three ml syringe, and received into EDTA coated vials, and immediately were kept in iced container, the samples were centrifuged at 3000rpm for 20 minutes, and plasma was separated in aliquots and transferred into plane vials. Plasma samples were stored at -20 and -80◦C until analysis.

Table 2. Diets composition

Group	**A**	**B**	**C**
Raw Materials%			
Corn %	70	60.0	59.0
Wheat hull %	0	4.1	5.4
Groundnut cake %	14.3	10	10
Concentrate %	5	5	5
CalciumCarbonate%	10	10	10
Salt (Nacl) %	0.125	0.25	0.125
Methionine %	0.34	0.34	0.31
Lysine %	0.15	0.15	0.11
Mycofix %	0.1	0.1	0.1
Flaxseeds seeds%	----	10	----
Sunflower seeds %	----	----	10
Premix*	0.1	0.1	0.1

*Supplied per kilogram of diets: Vitamin A, 5000 IU; Vitamin D ,500 IU; Vitmin E, 5 IU; Vitmin K, 1 IU; Vitmin B , 1.5 mg, Vitmin B , 2.5mg, 1 2 Ca-pantothenate, 2.5mg, niacin acid, 10 mg; pyridoxine,3mg; biotin, 0.1mg; folicacid, 0.25mg; Vitmin B , 0.005mg. Supplied per kilogram 12 b of diets: MnSO . 7H O100mg,; FeSO . 7H O, 220mg; ZnSO . 7H O, 150mg; CuSO . 7H O, 20mg; KI, 2mg; Na SeO, 0.4 mg.

A*=Control group,* ***B****=10% flaxseeds supplemented group,* ***C****=10% sunflower supplemented group.*

Table 3. Nutritional values calculated

Parameter	**A**	**B**	**C**
Metabolic Energy (ME) Kcal/Kg	2729	2832	2821
Crude protein (C.P)%	17.25	18.13	18.24
Either Extract (E.E)%	5	6.4	6.2
Crude Fiber (C.F)%	4.2	4.3	4.1
Available phosphorus%	0.52	0.65	0.63
Calcium%	3.9	3.9	3.9

A*=Control group,* ***B****=10% flaxseeds supplemented group, C=10% sunflower supplemented group.*

❖ **Fatty Acids Analysis:**

Lipids were extracted in chloroform-methanol (2:1 v/v), according to the method of [38]. **(Table.4)** Methyl esters of the lipid extract were prepared according to [39].

Table 4. Fatty acids profile of control and experimental group diets

Fatty acid	**(A)**	**(B)**	**(C)**
(g/100 g total fatty acids)			
C16:0	20.6791	7.4539	5
C17:0	20.679	---	----
C18:0	----	----	2.21
SFA	43.53	11.4265	8.58

18:1 (n-9)	17.68	16.19	17.09
MUFA	17.68	16.22	17.09
PUFA	38.38	72.3778	74.35
C18:3 (*n*-3)	1.38	49.1068	8.45
Σ*n*-3	1.38	49.1068	8.45
C18:2 (*n*-6)	36.94	11.6735	65.9
C20:4 (*n*-6)	0.21	3.4817	1.37
Σ*n*-6	37.15	15.1552	67.27
PUFA/SFA	0.88	6.33	8.7
Σ*n*-3/Σ*n*-6	0.04	3.24	0.13

SFA= Saturated fatty acids, **MUFA**=Mono unsaturated fatty acids, **PUFA**=Poly unsaturated fatty acids, **C18:3 (n-3)** =Linolenic acid (omega-3), **C18:2 (n-6)** =Linoliec acid (omega-6), **C20:4 (n-6)** = Arachidonic acid (omega-6). **C16:0**= Palmitic acid (saturated), **C17:0** = Heptadecanoic acid (saturated), **C18:1 (n-9)** = Oleic acid (Omega-9.)

A=Control group, **B**=10% flaxseeds supplemented group, **C**=10% sunflower supplemented group.

❖ **Gas Chromatograph Analysis:**

Fatty acid composition was determined using (Shimadzu-2010) gas chromatograph, fitted with Flame ionization detector (FID). Separation of fatty acids was achieved using DB-WAX column, serial number (us6551263 H), of 0.25um film thickness, 30 meter length and 0.25 mm inner diameter. Nitrogen was used as a carrier gas, 20 µl of sample were injected using split less mode.

Fatty acids methyl esters were identified by comparison of retention times with mixed standard purchased from (Sigma Aldrich-Germany), and expressed as percentage detected of methyl esters.

❖ **Determination of plasma oxidative status:**

Enzymatic antioxidant determination:

Catalase reaction was determined using commercial kits (Nanjing Jiancheng, China), through spectrophotometric method, following the instructions of the manufacturer.

Basically, ammonium molybdate can pause H2O2 decomposition reaction catalyzed by catalase (CAT) immediately, residual H2O2 can react with ammonium molybdate to produce a yellowish complex. This latter enables the calculation of CAT activity by measuring OD value at 405 nm.

Superoxide dismutase (SOD) activity was determined using commercial kits (Nanjing Jiancheng, China) through ELISA method, the instructions of manufacturer was followed.

Mainly, superoxide dismutase (SOD) plays an important role in oxidation-antioxidation balance of organisms, this enzyme can remove superoxide anion radicals (O_2-) to protect cells from damage.

- **Non-Enzymatic antioxidants determination:**

Malondialdehyde (MDA) was determined using commercial kits (Nanjing Jiancheng, China) through spectrophotometric method, the instructions of manufacturer was followed.

Lipid hydroperoxide decomposition products can condensate with thiobarbituric acid (TBA) to produce a red compound with absorption peak at 532 nm.

Uric acid was determined using commercial kits (Biosystem, Spain) through spectrophotometric method described by [40] and [41]; where, a colored complex that can be measured at 546 nm, the instructions of manufacturer was followed.

The level of vitamin C was estimated by spectrophotometric method described by [42], and expressed as mg/dl of plasma.

Ascorbic acid is oxidized by copper to form dihydroscorbic acid. The product was treated with 2, 4 dinitrophenyl hydrazine to form tris 2, 4 dinitrophenyl hydrazine which undergoes rearrangement to form a colored product with maximum absorption at 520 nm in spectrophotometer (Unicam8625. UK)..

Mainly 0.5 ml of plasma, 0.5 ml of water and 1 ml of TCA were mixed thoroughly and centrifuged. To 1 ml of the supernatant, 0.2 ml of DTC reagent was added and incubated at 37°C for 3 hours. Then 1.5 ml of sulphuric acid was added, well mixed and the solution was allowed to stand for 30 minute at room temperature.

Vitamin E content was estimated by spectrophotometric method, described by [43], which involves the conversion of ferric ions to ferrous ions by α-tocopherol and the formation of red

colored complex with 2, 2 dipyridyl. Absorbance of chromophore was measured at 520 nm in the spectrophotometer.
Basically, 0.5 ml of plasma, 1.5 ml of ethanol was mixed and centrifuged. The supernatant was dried at 80°C for 3 hours. Then 0.2 ml of 2, 2 dipyridyl solution and 0.2 ml of ferric chloride solution were added, well mixed and 4 ml of butanol was added. The color developed was read at 520 nm in the spectrophotometer (Unicam 8625. UK). Values were read as mg/dl of plasma.

Standards of vitamin C and vitamin E, were obtained from (Sigma Aldrich), stock solution was prepared, then 5 different standards (5, 10, 15, 20 and 25 ppm), were made out of the stock solution.
Standard curves of vitamin C and vitamin E, were drawn, and the concentration of the mentioned vitamins in plasma were calculated.

Vitamin A content was estimated by spectrophotometric method, described by [44]. Where we measured 1 ml of the analyzed liquid in test-tube I (centrifugal) with a tight
Stopper and added 1 ml of KOH solution, plugged the tube and shaked vigorously for
1 minute. The tube was first heated in a water bath (60°C, 20 minutes), then cooled down in cold water. We added 1 ml of xylene, plugged the tube and shaked vigorously again for 1 minute,
the tube was centrifuged (1500×g, 10 minutes), the separated extract (Upper layer) collected
and transfered it to the test tube II made of "soft" (sodium) glass. We measured the absorbance A1 of the obtained extract at 335 nm against xylene. The extract was irradiated in the test tube II with UV light for 30 minutes, then we measured the absorbance A2 as following:

$$Cx = (A1 - A2) \bullet 22.23$$

Cx = concentration of vitamin A, A1= first read, A2= second read after 30 minutes.

22.23 is the multiplier based on the absorption coefficient of 1% solution of vitamin A (as retinol form) in xylene at 335 nm in a measuring cuvette with 1 cm thickness.

Statistical Analysis:

The data were analyzed by using Statistics-10 program designed for Windows. Differences between obtained values were carried out by analysis of variance (ANOVA) the LCD test was used for determining the significance level of at least $p<0.05$.

Results:

The fourth week showed significant ($p<0.02$) high concentration of catalase in group (B), compared to group (A), while there was no significant difference between group (B) and (C). By the end of the 8thweek, group (B) and group (C), showed significant ($p<0.03$) high level of plasma catalase compared to the control group (A).
With regard to SOD level, Group (B) and (C), recorded significant ($p<0.04$) high concentration at the 4th week, compared to the control group (A), and Group (B), at week 8th, remained on the top with significant ($p<0.04$) high concentration of plasma SOD, compared to the control group (A). See **Table 5.**

Table 5. Plasma enzymatic antioxidants concentration

Parameter	CAT (U/ml)		SOD (U/ml)	
Group	4th week	8th week	4th week	8th week
A	41.000^{B}±6.0431	40.667^{B}±7.8811	32.500^{B}±2.4710	33.267^{B}±2.8718
B	70.667^{A}±6.0431	78.333^{A}±7.8811	43.300^{A}±2.4710	45.000^{A}±2.8718
C	66.667^{A}±6.0431	67.333^{AB}±7.881	41.333^{A}±2.4710	41.267^{AB}±2.878

***A**: Control group, **B**: Fed 10% flax seeds. C: Fed 10% sunflower seeds, **CAT**= Catalase, **SOD**=Superoxide dismutase.*
Data are means ± standard error. Means in the same column followed by the same letters are not significantly different at ($p < 0.05$).

There was no significant difference in the MDA plasma concentrations in all groups, neither by the end of the 4th week nor by the end of 8th week.
The uric acid plasma concentration instead in group (B) and (C), was significantly high ($p<0.05$) at the 4th week, compared to the control group (A). By the end of the 8thweek, group (C) recorded significant ($p<0.05$) high concentration of uric acid compared to the control group (A), but the difference between group (B) and the control group was not significant, Table 6.

Table 6. Plasma non-enzymatic antioxidants concentration

Parameter	**MDA (mmol/L)**		**Uric Acid (mg/dl)**	
Group	4th week	8th week	4th week	8th week
A	10.333^{A}±1.5275	10.867^{A}±1.6871	9.143^{B}±1.0723	9.143^{B}±1.0723
B	9.333^{A}±1.5275	10.000^{A}±1.6871	11.400^{A}±1.2687	7.6667^{AB}±0.3834
C	9.667^{A}±1.5275	9.233^{A}±1.6871	11.900^{A}±1.2687	11.900^{A}±1.2687

***A**: Control group, **B**: Fed 10% flax seeds. C: Fed 10% sunflower seeds, **MDA=** Malondialdehyde*
Data are means ± standard error. Means in the same column followed by the same letters are not significantly different at ($p < 0.05$).

There was no significant difference in vitamin C plasma levels in all groups at the 4th week, the control group (A), at the 8th week, recorded slightly high concentration of vitamin C. but it was not significant compared to group (B) and (C).

The plasma concentration of vitamin E, was significantly ($p<0.02$) high in group (C), at the fourth week, compared to group (A) and (B), the same result was noticed by the end of the 8th week.

The sunflower supplemented group (C), and flax seeds supplemented group (B), recorded significant ($p<0.01$) and ($p<0.05$) high concentration of plasma vitamin A, respectively, compared to the control group (A), Table 7.

Table 7. Plasma non-enzymatic antioxidants concentration

Parameter	**Vitamin C (mg/dl)**		**Vitamin E (mg/dl)**		**Vitamin A (µmol/l)**	
Group	4th week	8th week	4th week	8th week	4th week	8th week
A	10.600^{A}±0.659	16.000^{A}±1.116	2.9091^{B}±0.277	3.1833^{B}±0.43	0.3433^{A}±0.1343	0.5200^{B}±0.132
B	11.000^{A}±0.659 0	15.200^{A}±1.116 5	2.9818^{B}±0.277 5	4.0000^{B}±0.430 8	0.2800A±0.134 3	1.0467^{A}±0.132 8
C	10.625^{A}±0.736	15.000^{A}±1.116	4.0091^{A}±0.277	5.4500^{A}±0.43	0.3333A±0.134 3	1.1667^{A}±0.132

***A**: Control group, **B**: Fed 10% flax seeds, C: Fed 10% sunflower seeds.*
Data are means ± standard error. Means in the same column followed by the same letters are not significantly different at ($p < 0.05$).

Discussion:

Catalase is the first enzyme to show alteration following induction of oxidative stress, [45] and [46].

Significant high catalase plasma level, was observed in the treated groups compared to the control. The alteration of catalase concentration was fluctuated, this agrees with what was reported by [47], in a study performed to evaluate the antioxidant enzymes activities of (Cyprinus carpio), and fed diet contained moderate level of sunflower seeds. This result could be attributed to the intracellular localization of this enzyme responsible for different responses to oxidative stress, with an activity mainly found in peroximes, [48]. Considering these fluctuating results, the values can reflect also an adaptation/acclimation to diet composition [49].

Tejaswi; *et al*., 2013), reported, normal level of serum catalase in rats dosed by paracetamol after 30 minutes of flaxseeds administration, in comparison with healthy and positive control (received Silymarin 30 minute before administration of paracetamol), these results corroborate our current result, that flaxseeds has potency to improve the oxidative status through elevating the levels of catalase enzyme. Superoxide dismutase, is the first enzyme to response against oxygen radicals [50]; and oxidative stress [51].

The result of this study revealed, significant high level of plasma SOD concentration in both, flax seeds and sunflower supplemented groups by the end of the first month and the same result was observed by the end of the second one, this result agrees with that reported by [52], when they conducted a trial to investigate the hepato-protective activity of omega-3 fatty acids obtained from flax seeds and fish oil, they stated that; administration of previously mentioned oil to rats, dosed by paracetamol repeatedly; achieved normalization of oxidative status, through improvement in levels of antioxidant enzymes and oxidative stress markers.

Postulations by [53], suggested that, polyunsaturated fatty acids (PUFA), can scavenge free radicals, improve the activity of SOD and other antioxidant enzymes, and may exert a preventive antioxidant role

against free radicals action. The fatty acids profile and the percentage of plasma polyunsaturated fatty acids in the treated groups, agree with the suggestions mentioned above.

Uric acid is an antioxidant, produced in vivo, primarily as product of the degradation of purine compounds, rather than consumed in diet [54].

The plasma concentration of uric acid at the fourth week, was significantly high in treated groups compared to the control group, this result could be attributed to high plasma triglycerides concentration in the treated groups at the first month, because plasma triglycerides is related to hyperuricemia [55] and [56]. Triglycerides synthesis needs great amounts of NADPH [57]; the synthesis of fatty acids in the liver is associated with the de novo synthesis of purines, thus accelerating uric acid production [58].

Also this result is in agreement with the findings of [59], who reported that, adding sunflower oil and corn oil to the diets of Japanese quail, resulted in significant elevation of plasma uric acid concentration, compared to fish oil and flax seeds oil, while the concentration of uric acid between the sunflower oil group and corn oil supplemented group was not significant.

Malondialdehyde, is the major oxidation product of per oxidized polyunsaturated fatty acids.

The current study, showed no significant different levels of plasma MDA, between the control group and the treated groups, these results go with the findings of [23], who studied the influence of dietary grill oil on blood and liver oxidants/antioxidants status, and the parameters of liver function in laying Japanese quail. Their results showed no significant influence of sunflower oil or grill oil on the level of serum MDA in a comparison with the control group.

Another corroborate to our results, was reported by [60], when he studied the anti-hyperglycemic effect of ethanol extract of sunflower seeds on normal and glucose loaded hyperglycemic, and streptozotocin induced type 2 diabetic rats and found significant reduction of serum MDA level in diabetic group which received ethanolic extract of sunflower seeds compared to the positive control (diabetic+glibenamide), and the difference was significantly low compared to the negative control group (diabetic rats not treated with glibenamide).

The biosynthesis of ascorbic acid in mammals and birds takes place in the liver/kidney or both.

In the chickens the synthesis occurs primarily in the kidneys as reported by [61], usually, sugars such as glucose, fructose and mannose, serve as precursors for vitamin C synthesis.

There was no significant different levels of vitamin C, between all groups at the second month, group A, showed slightly higher levels, this may be due to the capability of hens to produce the vitamin de novo at the kidney and liver, and as time passing by, the concentration of vitamin C in plasma increased due to individual variation of ascorbic acid synthesis, or it could be attributed to the slightly higher sunflower seeds content of vitamin C, also the reverse relationship between vitamin C and uric acid could be a justification.

Vitamin **E**, is the primary lipid soluble anti-oxidant, found in food and human blood and tissues, it is well known that vitamin E, inhibits the process of lipid peroxidation in oils and in the biological lipid-protein complexes, such as biological membranes or circulating lipoproteins [62].

Group C, which received 10% sunflower seeds, showed significant high concentration of plasma vitamin E, compared to the control group and group B, the high concentration of vitamin E in group C plasma, is a reflection of the high vitamin E content in group C diet, this result is in agreement with what was reported by [63], that sunflower seeds oil is considered as a rich source of vitamin E.

Vitamin A was measured as biomarkers for oxidative stress that could be associated with consumption of diets rich in PUFA, in the present study the plasma concentration of vitamin A was significantly increased in response to sunflower seeds addition compared to the control group, this result concurred with [23], who reported significant decrease in plasma vitamin A level when Japanese quail fed diet containing sunflower oil. According to [64], sunflower seeds contains 50 IU/100 g of vitamin A, so the opposite result in the present study could be attributed to the fact that vitamin A in sunflower oil is oxidized and highly exposed to the environmental conditions, while the whole sunflower seed is naturally protected from the oxidation factors.

In the present study the plasma concentration of vitamin A was significantly increased in response to flax seeds addition compared to the control group, this result does not agree with [23], who reported significant decrease in plasma vitamin A level when Japanese quail fed diet containing sunflower oil and grill oil, they assumed that n-3 fatty acids and vitamin E have opposite effects on the metabolic regulation of absorption and/or conversion of β-carotene to retinol in plasma and platelets. The contrary result in the present study could be attributed to the difference of omega-3 supplementation source, or it could be attributed to the different kinds of birds used and their endogenous ability to improve their internal oxidative status, and their capability to absorb and convert β-carotene to retinol in plasma and platelets

Conclusion

The results obtained from the current study appointed that, feeding laying hens 10% sunflower and 10% flax seeds for 8 weeks, enhance the oxidative status, without any elevation of plasma **MDA**, though the high content of **PUFA** in the experimental diet. The improvement in oxidative status in return will be reflected in improvement of laying hen health and production.

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