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Effect of Different Levels of Sunflower Meal and Niacin on Performance, Biochemical Parameters, Antioxidant Status, and Egg Yolk Cholesterol of Laying Hens

Research Article

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ABSTRACT

This study was conducted to evaluate the effect of different levels of sunflower meal (SFM) and niacin on laying hens performances, biochemical parameters, antioxidant status, and egg yolk cholesterol concentration. A total of 960, White Leghorn (Hy-Line W-36) commercial layers were randomly assigned to 12 groups-of 8 replicates with 10 hens each. Hens were allocated to diets 1 through 12 in a 3 × 4 factorial design and the dietary treatments included 3 levels of SFM (0, 10 and 15%) and 4 levels of niacin (0, 175, 225 and 275 mg/kg) fed to the birds for 10 weeks. Feed consumption was not affected (P>0.05) by niacin, SFM and SFM × niacin interaction. Dietary supplementation of 15% of SFM significantly (P<0.05) reduced egg weight, egg production and consequently egg mass. However, dietary addition of 275 mg of niacin/kg of diet increased (P<0.05) egg production and egg mass. Egg weight was not affected (P>0.05) by dietary addition of niacin. Feed conversion ratio was affected (P<0.05) by dietary addition of SFM and was not affected (P>0.05) by dietary incorporation of niacin and the SFM × niacin interaction. In fact, dietary supplementation of 15% of SFM significantly increased (P<0.05) FCR from 2.12 to 2.14. With the exception of shell thickness, all other parameters were not affected (P>0.05) by dietary addition of niacin. Dietary supplementation of 15% of SFM reduced (P<0.05) egg shell thickness from 0.29 to 0.28. However, only egg shell strength was affected (P<0.05) by dietary incorporation 275 mg of niacin/ kg of diet. Egg yolk cholesterol content was affected (P<0.05) by dietary addition of SFM and niacin. Our data also showed that serum concentration of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and triglycerides (TG) levels were not affected (P>0.05) by dietary incorporation of SFM, niacin, and their interaction. By contrast serum concentration of total cholesterol (TC) were affected (P<0.05) by dietary addition of SFM, niacin and their interaction. There were no effects (P>0.05) of the experimental diets on total antioxidant capacity (T-AOC), total superoxide dismutase (TSOD), glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) serum concentration.

KEY WORDS laying hens, niacin, performance, shell thickness, sunflower meal.

INTRODUCTION

Recently, the consumer's over demand and high prices for other livestock meat have created a specialized business for poultry products (chicks, egg and broiler). The main constraint facing the poultry industry is the cost of feed, which approximately represents 75% of the total cost of production. Therefore, considerable interest is being shown in the formulation of high efficiency poultry diets based on the utilization of locally available feed ingredients to minimize

the cost of poultry feeding (Abdalla, 1999). Sunflower (*Helianthus annuus*) is one of the most important oil crops. Sunflower seeds are important sources of oil and protein, it contained high content of oil and reached up to 45% (Baghban-Kanani *et al.* 2018). As the cultivation of soybean is limited in Iran, a large amount of soybean meal is annually imported. Recently, cultivation of some oil seeds, such as sunflower, is undertaken in some provinces of Iran (Rezaei and Hafezian, 2007). Sunflower seed can be harvested two or three times a year in tropical areas and is a good alternative for oil producers and for feed mill sector. Feeding value of sunflower for poultry is expected to be affected by the variety used and the soil under which it is grown. Most of the studies performed to evaluate the use of sunflower in hen feeding showed that a high level of sunflower meal can be incorporated in the laying hen's diet without any negative effect on performance and egg quality (Tsuzuki *et al.* 2003). Sunflower meal contains substantial concentrations of cell-wall material and a high fiber content that could reduce cholesterol. Baghban-Kanani *et al.* (2018) reported that dietary supplementation of SFM up to 20% together with a multi-enzyme complex did not appear to cause any adverse effects on egg production and quality as well on antioxidant status in laying hens (Baghban-Kanani *et al.* 2018). Niacin, a water-soluble vitamin, is an essential nutrient which is also known as vitamin B_3 or vitamin PP. It exists as nicotinic acid and nicotinamide which have equal biological activity and can be synthesized from tryptophan. The terms niacin, nicotinamide, and vitamin B_3 are often used interchangeably to refer to any member of this family of compounds (Lawrance, 2015). Niacin is directly or indirectly involved in many metabolic functions including the digestive system, skin, and nerves. These coenzymes are involved in transfers of hydrogen, which frequently occur in the synthesis and degradation of fatty acids, carbohydrates and amino acids. It is also important for converting food to energy (Lawrance, 2015). Due to niacin antagonists in the diet and the bound structure in grains and other feedstuffs, higher doses of supplemental niacin may be needed for optimal production. NRC (1994) set the niacin requirement for laying hens as 10.0 mg/kg. Since niacin absorption and utilization are affected by many factors, including complexion of niacin with macromolecules in cereals, tryptophan and vitamin B_6 levels in the diet, higher supplementation levels than the presently accepted requirement may be needed for laying hens (Gungor *et al.* 2003). The effects of niacin on serum and egg cholesterol concentrations have been reported by some researchers in several animal species. Kurtoglu *et al.* (2004) reported that dietary supplementation of 50 to 300 ppm of niacin significantly decreased egg yolk and serum cholesterol concentrations. Therefore, the objective of this study was to evaluate the interaction of dietary supplementation of different levels of sunflower meal and niacin on laying hen's performances, biochemical parameters and egg yolk cholesterol concentrations.

MATERIALS AND METHODS

Procedures related to animals care, handling and sampling were conducted under the approval of Institutional Animal Care and Use Committee of Tabriz University (Tabriz-Iran).

Birds and experimental design

Sunflower meal chemical composition is presented in Table 1. Metabolizable energy content of sunflower meal was estimated using the following equation (NRC, 1994):

$$MEn = 26.7 \times DM + 77 \times EE - 51.22 \times CF$$

Where:
DM: % of dry matter.
EE: % of Ether extract.
CF: % of Crude Fiber of sunflower.

Nine hundred and sixty, 55 weeks old, Hy-Line W-36 White Leghorns laying hens were used in 12 treatments with 8 replicates and 10 hens in each treatment in a factorial arrangement 3×4 by completely randomized design (Table 2). Hens were housed in individual cages with (41×23×43 cm), in a room with ambient temperature of about 20 ˚C and a photoperiod of 16 h light: 8h darkness cycle. Feed and water were offered *ad-libitum* during the experimental period. The experiment lasted 12 weeks. Diets were formulated according to the basis of linear programming using UFFDA software. Diets were prepared by mixing a control diet based on corn and soybean-meal thoroughly with the designated supplements: three levels (0, 10 and 15%) of sunflower meal and four levels of Niacin 99% (0, 175, 225 and 275 mg/kg). Diets composition was shown in Table 3.

Sample collection

Birds were weighted at the beginning and the end of the experiment to determine the live weight changes. Laying hens performances (feed intake, egg weight, egg mass, egg production and feed conversion ratio) were determined per week. Feed efficiency was calculated as the rate of feed consumption and the number of eggs × egg weight. Eggs laid during the week were used for egg specific gravity and Haugh unit measurements. At the end of the experiment, a total of 240 eggs from each treatment were collected and used for egg yolk cholesterol content determination (Elkin and Rogler, 1990).

Table 1 Chemical composition and metabolizable energy content of high fiber sunflower meal (%)

Nutrients	Dry matter	Crude protein	Ether extract	Crude fiber	Ash	NFE[1]	MEn[2] (kcal/kg)	Ca[3]	P[4]	Na[5]
	93.40	19.10	8.48	25.40	7.58	39.44	1845.76	0.51	0.82	0.22

NFE: nitrogen free extract; MEn: nitrogen-corrected metabolizable energy; Ca: calcium; P: phosphorus and Na: natrium.

Table 2 Experimental design

Treatment	Sunflower meal (%)	Niacin (mg/kg)	Treatment	Sunflower meal (%)	Niacin (mg/kg)
T1	0	0	T7	10	225
T2	0	175	T8	10	275
T3	0	225	T9	15	0
T4	0	275	T10	15	175
T5	10	0	T11	15	225
T6	10	175	T12	15	275

Table 3 Composition of the experimental basal diet

Ingredients	(%)
Corn	61.46
Soybean meal (44 %)	23.56
Oyster mineral	9.67
Veg oil	2.40
Dicalcium phosphate	1.88
Vitamin premix[1]	0.25
Mineral premix[2]	0.25
Salt	0.20
Dl-methionine	0.30
Threonine	0.03
Chemical composition (%)	
Nitrogen-corrected metabolizable energy (AME_n, kcal/kg)	2830
Crude protein	15.25
Ether extract	3.01
Crude fiber	2.78
Calcium	4.35
Available phosphorus	0.46
Sodium	0.11
Potassium	0.61
Methionine	0.52
Methionine + cistine	0.67
Lyine	0.78
Arginine	0.97

[1] Vitamin supplement provides per kilogram of diet: vitamin A: 8000 IU; vitamin E: 20 IU; Menadione: 3.0 mg; vitamin D_3: 2000 IU; Riboflavin: 4.0 mg; Ca-pantothenate: 12 mg; Nicotinic acid: 50 mg; Choline: 300 mg; vitamin B_{12}: 15 mg; vitamin B_6: 0.12 mg; Thiamine: 1.5 mg; Folic acid: 1.00 mg and Biotin: 0.10 mg.
[2] Mineral supplement provides per kilogram of diet: Trace mineral (milligrams per kilogram of diet): Mn: 100 mg; Zn: 70 mg; Fe: 50 mg; Cu: 10 mg; Iodine: 1 mg; Se: 0.30 mg and Antioxidant: 50 mg.

Samples were stored at -80 ˚C for further analyses. Blood samples of ten hens from each replicate were collected from wing vein into additive free blood tubes. Samples were centrifuged at 4000 (rpm) for 10 min at +20 ˚C. Serum was then used for antioxidant capacity, alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglycerides (TG), total cholesterol (TC) and malondialdehyde (MDA) determination.

Antioxidant status parameters

Serum samples were used for antioxidant capacity, including total antioxidant capacity (T-AOC), total superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) determination using RANDOX kits (Germany). Determination of serum SOD activity was conducted using the xanthine oxidase methodology reported by (Winterbourn *et al.* 1975).

In fact, this procedure monitored the degree of inhibition of nitroblue tetrazolium reduction by O_2-generated by xanthine and xanthine oxidase. Absorbance was measured at 550 nm using a spectrophotometer (UV-1201, Shimadzu, Japan). Serum lipid peroxidation (LP) was determined using the 1,1,3,3-tetraethoxypropane as a standard as reported by Satoh (1978) and Yagi (1984).

In fact, MDA (aldehyde lipid peroxidation) product formed a pink-colored complex with thiobarbituric acid (TBA). Absorbance was measured at 532 nm using a spectrophotometer (UV-1201, Shimadzu, Japan). The Serum LP values were expressed as MDA nmol/mL of plasma.

Statistical analyses

Data were subjected to one-way ANOVA with 12 treatments and 8 replicates with 10 hens in each replicate, using the general linear model (GLM) procedure of SAS software (SAS, 2003) for windows. Means were compared using Duncan's multiple range tests at 5% probability.

RESULTS AND DISCUSSION

Effects of experimental diets on laying hens' performances and egg parameters are shown in Table 4. Feed consumption was not affected (P>0.05) by niacin, SFM and SFM × niacin interaction. Dietary supplementation of 15% SFM significantly (P<0.05) reduced egg weight, egg production and consequently egg mass. However, dietary addition of niacin increased (P<0.05) egg production from 81.22% (0%) to 82.13%, 82.83% and 83.15% for respectively 175, 225 and 275 mg niacin/kg. In parallel, egg mass increased (P<0.05) from 49.22 g/bird/day (0%) to 50.68 g/bird/day (275 mg/kg). Egg weight was not affected (P>0.05) by dietary addition of niacin. Concerning SFM × niacin interaction, only egg production and mass were significantly affected (P<0.05) by this interaction. T3 and T4 had the highest egg production and mass. However, T9 had the lowest values. FCR was affected (P<0.05) by dietary addition of SFM and was not affected (P>0.05) by dietary incorporation of niacin and the SFM × niacin interaction. In fact, dietary supplementation of 15% of SFM significantly increased (P<0.05) FCR from 2.12 to 2.14. Effects of experimental diets on egg quality parameters (shell thickness, shell strength, shape index, Haugh unit and egg specific gravity) are summarized in Table 5.

Our results showed that with the exception of shell thickness, all other parameters were not affected (P>0.05) by dietary addition of niacin. Dietary supplementation of 15% of SFM reduced (P<0.05) egg shell thickness from 0.29 to 0.28. However, only egg shell strength was affected (P<0.05) by dietary incorporation of niacin. In fact, egg shell strength increased (P<0.05) from 3.14 to 3.56 in response to added niacin at a level of 275 mg/kg. Effects of experimental diets on egg yolk weight and cholesterol content are presented in Table 6.

Egg yolk cholesterol content was affected (P<0.05) by dietary addition of SFM and niacin. The 15% of SFM was associated with the lowest egg yolk cholesterol content.

Compared to the control diet, dietary supplementation of 275 mg of niacin/kg reduced (P<0.05) from 11.87 to 10.95 mg/g of yolk corresponding to 206.62 mg/yolk and 191.53 mg/yolk. The SFM × niacin interaction was significant (P<0.05) for egg yolk cholesterol. T12 was associated with the lowest value of egg yolk cholesterol. Table 7 shows the effect of experimental diets on plasma biochemical parameters of laying hens. Our data showed that serum concentration of AST, ALT and TG levels were not affected (P>0.05) by dietary incorporation of SFM, niacin, and their interaction. By contrast serum concentration of TC were affected (P<0.05) of SFM, niacin and their interaction. The lowest plasma cholesterol levels were recorded with dietary supplementation of 15% of SFM (P<0.05) or niacin (P<0.05) at 275 mg/kg. Plasma cholesterol was also significantly affected (P<0.05) by SFM × niacin interaction. T12 was associated with the lowest mean values. Effects of dietary treatments on serum antioxidant status of laying hens are shown in Table 8. There were no effects (P>0.05) of the experimental diets on T-AOC, TSOD, GSH-Px and MDA serum concentration.

Dietary addition of SFM did not affect feed consumption. These results were not in agreement with those reported by Baghban-Kanani *et al.* (2018) who found that hens' fed on diets with 20% of SFM had lower feed intake without affecting feed efficiency. Supplementation of 15 % of SFM in the diet significantly reduced egg weight, egg production and mass. Consequently, feed conversion ratio significantly increased with the supplementation of 15% SFM. Our findings are in line with those of Rose *et al.* (1972) who reported a reduction in egg production and weight of hens fed on diets in which SFM protein was substituted for all the protein provided by soybean meal. Rezaei and Hafezian (2007) also found that dietary addition of 15% of SFM reduced egg production and weight. The most limiting nutrients in the diets with SFM used in this study were energy. Thus in diets containing more than 10% of SFM, laying hens probably did not get sufficient energy to maintain egg production. In addition, the fiber content of the diets may have contributed to the poor feed conversion of hens fed on SFM at different levels compared to the control diet. In contrast to our results, Shi *et al.* (2012) reported that laying hens' diets supplemented with 8.26, 16.52, and 24.84% of SFM had no significant effect on body weight gain, egg production, egg mass, feed intake and feed conversion. The differences between the present study and other experiments may be attributed to the differences in the laying period, age and genotype of hen and the combination and to the level dietary incorporation of SFM. In our study, dietary addition of 225 and 275 mg/kg of niacin significantly increased egg production and mass.

Table 4 Effect of experimental diets on laying hens performances and egg parameters

Item	Feed consumption (g/d/bird)	Egg production (%)	Egg weight (g)	Egg mass (g/d/bird)	FCR
SFM %					
0	109.66	84.14^{a}	61.28^{a}	51.56^{a}	2.12^{b}
10	108.52	82.10^{ab}	61.12^{a}	50.18^{ab}	2.16^{ab}
15	107.30	80.75^{b}	60.12^{b}	48.55^{b}	2.21^{a}
SEM	0.78	0.44	0.23	0.30	0.02
Niacin mg/kg					
0	108.05	81.22^{b}	60.59	49.22^{b}	2.19
175	108.57	82.13^{ab}	60.91	50.02^{ab}	2.17
225	109.03	82.83^{a}	60.91	50.47^{a}	2.16
275	108.05	83.15^{a}	60.94	50.68^{a}	2.14
SEM	0.91	0.50	0.27	0.35	0.02
SFM × niacin					
T1	108.90	81.54^{bc}	61.00	49.74^{bcd}	2.18
T2	109.90	83.59^{ab}	61.34	51.26^{ab}	2.14
T3	109.91	85.23^{a}	61.38	52.32^{a}	2.10
T4	109.91	86.20^{a}	61.38	52.91^{a}	2.07
T5	109.22	81.80^{bc}	61.03	49.93^{bcd}	2.18
T6	108.37	82.12^{bc}	61.14	50.21^{bc}	2.15
T7	108.86	82.25^{bc}	61.12	50.26^{bc}	2.16
T8	107.62	82.23^{bc}	61.19	50.32^{bc}	2.13
T9	106.03	80.32^{c}	59.74	47.99^{d}	2.21
T10	107.44	80.67^{bc}	60.24	48.59^{cd}	2.21
T11	108.31	81.02^{bc}	60.24	48.81^{cd}	2.22
T12	107.42	81.01^{bc}	60.24	48.80^{cd}	2.20
SEM	1.57	0.88	0.47	0.61	0.04
P-value					
SFM	0.12	0.0001	0.002	0.0001	0.04
Niacin	0.89	0.05	0.77	0.03	0.51
SFM × niacin	0.82	0.0005	0.20	0.0001	0.48

T1 (control): diet without sunflower meal and niacin supplementation; T2: control diet supplemented with 175 mg of niacin/kg of diet; T3: control diet supplemented with 225 mg of niacin/kg of diet; T4: control diet supplemented with 275 mg of niacin/kg of diet; T5: control diet supplemented of 10% of sunflower meal; T6: control diet supplemented of 10% of sunflower meal and 175 mg of niacin/kg of diet; T7: control diet supplemented of 10% of sunflower meal and 225 mg of niacin/kg of diet; T8: control diet supplemented of 10% of sunflower meal and 275 mg of niacin/kg of diet; T9: control diet supplemented of 15% of sunflower meal; T10: control diet supplemented of 15% of sunflower meal and 175 mg of niacin/kg of diet; T11: control diet supplemented of 15% of sunflower meal and 225 mg of niacin/kg of diet and T12: control diet supplemented of 15% of sunflower meal and 275 mg of niacin/kg of diet.
FCR: feed conversion ratio; SFM: sunflower meal and SFM × niacin: sunflower meal and niacin interaction.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

These results are in agreement with the findings of Kurtoglu *et al.* (2004) who reported that dietary supplementation of 200 and 250 ppm niacin during a 60-90 day period increased egg production from 80% (control) to 83.94% and 85.31% for respectively, 200 and 250 ppm. Triebel (1981) also reported that dietary supplementation of 25 to 75 ppm niacin enhanced laying hens' egg production and weight.

These beneficial effects of niacin on egg characteristics were not correlated with increases in feed intake. Gungor *et al.* (2003) found that supplemental niacin up to 500 mg/kg increased egg production when compared to the control diet and dietary addition of 1500 mg of niacin/kg was associated to the highest egg production.

Niacin could increase egg production through providing energy from carbohydrates, fats and proteins.

An increase of utilization of calcium and phosphorus may be attributed to the niacin. In this regard, El-Husseiny *et al.* (2008) reported that dietary supplementation of niacin in layer diets improved egg production and quality.

In our trial, shell thickness decreased in response to added 15% of SFM, when compared to the control groups of hens. In contrast to these results found in the present study, Baghban-Kanani *et al.* (2018) and Shi *et al.* (2012) reported no negative effects on egg quality of hens' fed on SFM.

Nutrients intake, especially calcium and phosphorus, by hens fed low energy rations with crude fiber levels higher than 10% was not sufficient to maintain thickness of egg shell. Dietary supplementation of 225 and of 275 mg/kg niacin increased egg shell strength compared to the control group.

Table 5 Effect of experimental diets on egg quality of laying hens

Item	Shell thickness (mm)	Eggshell strength (kg/cm^2)	Shape index (%)	Haugh unit	Egg specific gravity (g/cm^3)
SFM %					
0	0.29^{a}	3.54	74.19	77.66	1.06
10	0.28^{ab}	3.35	73.89	77.19	1.06
15	0.28^{b}	3.33	73.56	77.02	1.06
SEM	0.003	0.09	0.49	1.03	0.001
Niacin mg/kg					
0	0.28	3.14^{b}	73.47	77.15	1.06
175	0.28	3.37^{ab}	73.76	77.31	1.06
225	0.29	3.56^{a}	74.12	77.41	1.06
275	0.29	3.56^{a}	74.17	77.38	1.06
SEM	0.004	0.11	0.59	1.00	0.002
SFM × niacin					
T1	0.28	3.13	73.32	77.10	1.06
T2	0.29	3.55	74.27	77.63	1.06
T3	0.30	3.72	74.52	78.01	1.06
T4	0.30	3.74	74.60	77.91	1.06
T5	0.28	3.14	73.82	77.21	1.06
T6	0.28	3.15	73.67	77.19	1.06
T7	0.28	3.56	73.80	77.17	1.06
T8	0.29	3.57	74.09	77.18	1.06
T9	0.27	3.13	73.28	77.12	1.06
T10	0.27	3.40	73.38	77.10	1.06
T11	0.28	3.40	73.80	77.06	1.06
T12	0.28	3.41	73.82	77.07	1.06
SEM	0.007	0.19	0.98	2.06	0.002
P-value					
SFM	0.007	0.27	0.66	0.91	0.80
Niacin	0.59	0.03	0.80	0.99	0.98
SFM × niacin	0.24	0.23	0.99	1.00	0.99

T1 (control): diet without sunflower meal and niacin supplementation; T2: control diet supplemented with 175 mg of niacin/kg of diet; T3: control diet supplemented with 225 mg of niacin/kg of diet; T4: control diet supplemented with 275 mg of niacin/kg of diet; T5: control diet supplemented of 10% of sunflower meal; T6: control diet supplemented of 10% of sunflower meal and 175 mg of niacin/kg of diet; T7: control diet supplemented of 10% of sunflower meal and 225 mg of niacin/kg of diet; T8: control diet supplemented of 10% of sunflower meal and 275 mg of niacin/kg of diet; T9: control diet supplemented of 15% of sunflower meal; T10: control diet supplemented of 15% of sunflower meal and 175 mg of niacin/kg of diet; T11: control diet supplemented of 15% of sunflower meal and 225 mg of niacin/kg of diet and T12: control diet supplemented of 15% of sunflower meal and 275 mg of niacin/kg of diet.
SFM: sunflower meal and SFM × niacin: sunflower meal and niacin interaction.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

In this respect, Gungor *et al.* (2003) found that addition of niacin at 250 and 500 mg/kg levels resulted in a significant increase in egg shell strength. Niacin deficiency results in poor utilization of calcium and phosphorus which are required for normal egg and bone formation (El-Husseiny *et al.* 2008).

Niacin increases utilization of calcium and phosphorus that are necessary for egg strength. In the present study, hens fed on 15% SFM or 275 mg/kg niacin had the lowest egg yolk and plasma cholesterol concentration. These results are in agreement with the findings of Baghban-Kanani *et al.* (2018) who found a significant decrease in egg yolk cholesterol concentration in response to the dietary addition of 20% of SFM.

Shi *et al.* (2012) showed that substation of soybean meal with different levels of SFM decreased egg yolk cholesterol.

These results might be explained by the fact that cholesterol intake from a conventional laying hens diet is minimal, and endogenous synthesis in hen is absolutely required for providing the cholesterol needed for eggs, or used as a structural component of cell membranes and as precursor to sex and adrenal hormones, vitamin D and the bile acids (Kurtoglu *et al.* 2004).

The liver and the ovary are the primary sites of cholesterol biosynthesis in the laying bird. However, the liver is the major source of most lipids found in egg yolk. The use of sunflower meal can be beneficial due to the effect of fiber reducing cholesterol.

One mechanism through which sunflower meal may exert its hypo-cholesterolemic action is via bile acids. The cholic and deoxycholic bile acids are produced from cholesterol by hepatocytes and are conjugated with glycine and taurine, respectively.

Table 6 The effects of experimental diets on yolk weight and yolk cholesterol

Item	Yolk weight (g)	Yolk cholesterol (mg/yolk)	Yolk cholesterol (mg/g of yolk)
SFM %			
0	17.75	209.37^{a}	11.89^{a}
10	17.70	202.91^{ab}	11.57^{ab}
15	17.41	186.41^{b}	10.80^{b}
SEM	0.16	2.93	0.17
Niacin mg/kg			
0	17.55	206.62^{a}	11.87^{a}
175	17.64	201.93^{ab}	11.53^{ab}
225	17.64	198.17^{ab}	11.32^{ab}
275	17.65	191.53^{b}	10.95^{b}
SEM	0.19	3.38	0.20
SFM × niacin			
T1	17.66	217.18^{a}	12.39^{a}
T2	17.77	209.90^{ab}	11.91^{ab}
T3	17.79	207.92^{abc}	11.77^{ab}
T4	17.78	202.48^{abc}	11.47^{ab}
T5	17.69	212.32^{ab}	12.12^{ab}
T6	17.70	205.85^{abc}	11.71^{ab}
T7	17.70	201.26^{abc}	11.47^{ab}
T8	17.72	192.21^{abc}	10.98^{ab}
T9	17.30	190.37^{abc}	11.11^{ab}
T10	17.44	190.03^{abc}	10.99^{ab}
T11	17.44	185.33^{bc}	10.71^{ab}
T12	17.44	179.91^{c}	10.41^{b}
SEM	0.33	5.86	0.34
P-value			
SFM	0.30	0.0001	0.0003
Niacin	0.97	0.02	0.02
SFM × niacin	0.99	0.0007	0.007

T1 (control): diet without sunflower meal and niacin supplementation; T2: control diet supplemented with 175 mg of niacin/kg of diet; T3: control diet supplemented with 225 mg of niacin/kg of diet; T4: control diet supplemented with 275 mg of niacin/kg of diet; T5: control diet supplemented of 10% of sunflower meal; T6: control diet supplemented of 10% of sunflower meal and 175 mg of niacin/kg of diet; T7: control diet supplemented of 10% of sunflower meal and 225 mg of niacin/kg of diet; T8: control diet supplemented of 10% of sunflower meal and 275 mg of niacin/kg of diet; T9: control diet supplemented of 15% of sunflower meal; T10: control diet supplemented of 15% of sunflower meal and 175 mg of niacin/kg of diet; T11: control diet supplemented of 15% of sunflower meal and 225 mg of niacin/kg of diet and T12: control diet supplemented of 15% of sunflower meal and 275 mg of niacin/kg of diet.
SFM: sunflower meal and SFM × niacin: sunflower meal and niacin interaction.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

These acids enter the small intestine, where they are absorbed and directed to liver, and a decrease in bile acid recycling would ultimately result in a lowering of serum cholesterol concentration because cholesterol is used for bile acid synthesis (St-Onge *et al.* 2000). However, we did not measure the amount of bile acid synthesis to support this hypothesis.

Fiber content of sunflower meal may stimulate binding of cholesterol with bile acids, and inhibit micelle formation combined with the effect of fermentation on short chain fatty acid production. These are mechanisms that have been proposed to explain the potential cholesterol lowering effects (St-Onge *et al.* 2000). For gut bacterial fermentation to play a role in the hypolipidaemic effect of a food, sufficient propionate must be produced to offset the effects of acetate generation as a precursor for lipid synthesis (Jenkins *et al.* 1991).

An increase in bacterial count or a change in the composition of the bacterial population in the large intestine would result in increased fermentation and short chain fatty acid (SCFA) production. Depending on the proportion of each fatty acid produced, plasma cholesterol concentrations may thus be altered through this mechanism. Niacin would participate to efficiently reduce liver HMG-CoA reductase activity through its oxidative form ($NAD(P)^{+}$) and consequently would decrease the cholesterol deposition in the egg yolk. Vitamin B, especially niacin, in sunflower meal plays an important role in increasing high-density lipoprotein (HDL) and decreasing low-density lipoprotein (LDL) cholesterol. Sunflower meal is higher in niacin, riboflavin, choline, biotin, pantothenic acid and pyridoxine (Baghban-Kanani *et al.* 2018). Niacin intensified fatty acid oxidation would provoke accumulation of acetyl-CoA which in turn inhibits cholesterol synthesis.

Table 7 Effect of experimental diets on serum biochemical parameters of laying hens

Item	AST (U/L)	ALT (U/L)	TC (mg/dL)	TG (mg/dL)
SFM %				
0	214.79	4.86	100.32[a]	97.66
10	213.67	4.60	97.52[ab]	96.31
15	213.53	4.57	90.97[b]	93.96
SEM	0.49	0.16	1.93	1.93
Niacin mg/kg				
0	215.15	4.95	101.41[a]	98.10
175	214.13	4.66	96.66[ab]	96.36
225	213.50	4.58	94.87[ab]	95.31
275	213.21	4.51	92.14[b]	94.11
SEM	0.57	0.19	2.23	2.23
SFM × niacin				
T1	216.37	5.45	106.74[a]	100.93
T2	215.57	4.71	101.17[ab]	97.19
T3	213.49	4.67	98.67[ab]	96.92
T4	213.73	4.60	94.67[ab]	95.60
T5	214.90	4.69	102.43[ab]	97.80
T6	213.05	4.61	96.66[ab]	97.01
T7	213.61	4.59	96.50[ab]	95.59
T8	213.12	4.51	94.52[ab]	94.84
T9	214.17	4.72	65.06[ab]	95.57
T10	213.77	4.67	92.13[ab]	94.96
T11	213.39	4.48	89.45[ab]	93.43
T12	212.80	4.42	87.23[b]	91.90
SEM	0.99	0.33	3.86	3.86
P-value				
SFM	0.15	0.41	0.005	0.40
Niacin	0.10	0.38	0.04	0.63
SFM × niacin	0.32	0.80	0.05	0.96

T1 (control): diet without sunflower meal and niacin supplementation; T2: control diet supplemented with 175 mg of niacin/kg of diet; T3: control diet supplemented with 225 mg of niacin/kg of diet; T4: control diet supplemented with 275 mg of niacin/kg of diet; T5: control diet supplemented of 10% of sunflower meal; T6: control diet supplemented of 10% of sunflower meal and 175 mg of niacin/kg of diet; T7: control diet supplemented of 10% of sunflower meal and 225 mg of niacin/kg of diet; T8: control diet supplemented of 10% of sunflower meal and 275 mg of niacin/kg of diet; T9: control diet supplemented of 15% of sunflower meal; T10: control diet supplemented of 15% of sunflower meal and 175 mg of niacin/kg of diet; T11: control diet supplemented of 15% of sunflower meal and 225 mg of niacin/kg of diet and T12: control diet supplemented of 15% of sunflower meal and 275 mg of niacin/kg of diet.
SFM: sunflower meal and SFM × niacin: sunflower meal and niacin interaction.
AST: aspartate aminotransferase; ALT: alanine aminotransferase; TC: total cholesterol and TG: triglycerides.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 8 Effect of experimental diets on serum antioxidant status of laying hens

Item	MDA (nmol/mL)	TAC (U/mL)	TSOD (U/mL)	GSH (U/mL)
SFM %				
0	7.87	5.33	152.46	869.41
10	7.37	5.66	154.96	870.56
15	7.11	5.74	154.63	870.22
SEM	0.33	0.18	2.03	1.90
Niacin mg/kg				
0	8.08	5.49	153.34	865.16
175	7.43	5.39	153.33	870.20
225	7.19	5.63	154.17	871.46
275	7.09	5.71	154.84	872.35
SEM	0.38	0.20	2.34	2.20
SFM × niacin				
T1	8.49	4.88	151.09	864.65
T2	7.96	5.32	153.59	869.65
T3	7.57	5.62	152.59	871.46
T4	7.45	5.52	152.61	872.04
T5	7.90	5.80	154.58	866.41
T6	7.40	5.20	153.59	871.30
T7	7.17	5.55	155.08	871.56
T8	7.02	5.70	156.57	872.98
T9	7.86	5.78	154.34	864.54
T10	6.94	5.65	152.81	870.65
T11	6.84	5.72	154.84	872.41
T12	6.80	5.70	155.34	873.04
SEM	0.66	0.36	4.06	3.81
P-value				
SFM	0.27	0.26	0.66	0.88
Niacin	0.27	0.71	0.96	0.11
SFM × niacin	0.79	0.77	0.98	0.80

T1 (control): diet without sunflower meal and niacin supplementation; T2: control diet supplemented with 175 mg of niacin/kg of diet; T3: control diet supplemented with 225 mg of niacin/kg of diet; T4: control diet supplemented with 275 mg of niacin/kg of diet; T5: control diet supplemented of 10% of sunflower meal; T6: control diet supplemented of 10% of sunflower meal and 175 mg of niacin/kg of diet; T7: control diet supplemented of 10% of sunflower meal and 225 mg of niacin/kg of diet; T8: control diet supplemented of 10% of sunflower meal and 275 mg of niacin/kg of diet; T9: control diet supplemented of 15% of sunflower meal; T10: control diet supplemented of 15% of sunflower meal and 175 mg of niacin/kg of diet; T11: control diet supplemented of 15% of sunflower meal and 225 mg of niacin/kg of diet and T12: control diet supplemented of 15% of sunflower meal and 275 mg of niacin/kg of diet.
SFM: sunflower meal and SFM × niacin: sunflower meal and niacin interaction.
MDA: malondialdehyde; TAC: total antioxidant capacity; TSD: total superoxide dismutase and GS: glutathione peroxidase.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

On the other hand, NAD^+ or acetyl-CoA would also activate 7-α-hydroxylase, which allows biliary acid formation from cholesterol and would amplify its catabolism and excretion.

CONCLUSION

It was concluded that dietary addition of 225 and 275 mg/kg niacin improved egg production and egg shell strength. In addition, feeding laying hens with diets containing a combination of 15% SFM and of 275 mg/kg niacin decreased plasma and egg yolk cholesterol level.

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