Research Note

Effect of feeding low-fiber fraction of air-classified sunflower (*Helianthus annus* L.) meal on laying hen productive performance and egg yolk cholesterol

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ABSTRACT The present study was designed to determine the effect on laying performance and egg quality resulting from total substitution of soybean meal (SBM) with low-fiber sunflower meal (SFM; *Helianthus annus* L.) meal in diet of hens. ISA Brown layers, 28 wk of age, were randomly allocated to 2 dietary treatments and fed for 10 wk. The hens were kept in a free-range environment and fed 2 wheat middling-based diets consisting of a control diet, which contained SBM (153 g/kg of diet), and a test diet containing low-fiber SFM (160 g/kg of diet) as the main protein source. Each dietary treatment was replicated 4 times. Low-fiber SFM was obtained by a combination of sieving and air classification processes. Feed consumption was recorded daily and egg production was calculated on a hen-day basis; eggs from each group were collected weekly to evaluate egg components and quality. The total substitution of SBM with low-fiber SFM had no adverse effect on growth performance of laying hens. Egg production and none of egg quality traits examined were influenced by dietary treatment, except for yolk color ($P < 0.05$) and percentage of large-size eggs ($P < 0.05$) that were improved in hens fed the low-fiber SFM diet. Including low-fiber SFM decreased serum and egg yolk total cholesterol and low-density lipoprotein cholesterol concentrations ($P < 0.001$), and increased high-density lipoprotein cholesterol level. Our results suggest that the replacement of conventional soybean with low-fiber sunflower meal may be a valid alternative in diets for laying hens to improve egg quality and to develop low-cholesterol eggs.

Key words: sunflower meal, laying hen, air classification, egg quality, cholesterol

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INTRODUCTION

Soybean meal (**SBM**) is the most widely used protein source in the formulation of poultry diets. However, when the price of SBM increases, poultry nutritionists seek alternative protein sources that are more economical in formulating least cost rations. Sunflower (*Helianthus annus* L.) is a high oil-yielding seed crop cultivated worldwide that adapts very well to a wide range of climates; moreover, the meal is an important by-product as a source of vegetable protein and fiber for humans and animals (Kalmendal et al., 2011). Some limits of feeding sunflower meal (**SFM**) to monogastric species exist. Apart from lipid depletion during oil extraction, the relatively high fiber content of SFM places a penalty on its dietary energy content and limits its inclusion in poultry diets (Brenes et al., 2008), leading moreover to wet manure and dirty eggs. Dietary fiber may have functional properties, but cannot be digested by the endogenous enzymes of broilers and hens (Montagne et al., 2003), diluting the energy density of diets and also limiting feed intake (Zhou et al., 2013).

Reducing the SFM fiber content by processing could increase its dietary energy value and lead to greater inclusion levels in diet, and some promising results have been reported when cereals or legumes are sieved (Challa et al., 2010), pin milled (Wu and Nichols, 2005), or air-classified (Srinivasan and Singh, 2008; Zhou et al., 2013; Laudadio et al., 2014). Combining processes can be used to improve the enrichment of fractions. Thus, a combination of sieve fractionation and air classification can increase the degree of enrichment of the final fractions, in terms of higher protein concentration in finest fractions. Sieve fractionation combined with air classification increased protein content and decreased fiber content in SBM, wheat middlings, and barley (Challa et al., 2010; Srinivasan et al., 2012). However, in spite of these successes, to the best of our knowledge, the application of sieving air classification to SFM was never previously investigated.

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Therefore, the aim of this study was to evaluate the inclusion of low-fiber SFM as an alternative protein source to SBM in the laying-hen diet and to assess its effects on productive performance and egg quality.

MATERIALS AND METHODS

Experimental Birds and Management

This study was conducted in the experimental poultry facilities located at the University of Bari Aldo Moro, Italy, observing the animal welfare Legislative Decree 26/2014, Council Directive 99/74/CE, received in Italy by Legislative Decree 146/2001, and Council Directive 2010/63/CE, received in Italy by Legislative Decree 267/2003.

A total of 200 ISA Brown laying hens, 28 wk of age with an initial BW of 1,818 ± 11.9 g, were free-range reared for 10 wk. The hens were divided into 2 groups of 100 hens each (4 replicates each of 25 birds/group) and were housed in different indoor pens (0.15 m^2/bird) equipped with feeders and drinkers, with free access to open air runs (3 m^2/bird) without grass. The hens, before the start of the feeding trial, from 10 to 28 wk of age were free-range reared and fed a common corn-soybean-based diet.

Dietary Treatments

The hens were fed 2 experimental diets, a wheat middling-soybean diet (control) and wheat middling-sunflower-soybean diet. Each dietary treatment was replicated 4 times, with each replicate consisting of 1 pen of 25 birds. The diets (Table 1) were isocaloric and isonitrogenous containing 17.5% CP and 2,675 kcal of ME/kg of diet, designed to meet or exceed the nutrient requirements for laying hens (NRC, 1994). The ME of the basal diet was estimated using the Carpenter and Clegg equation (Leeson and Summers, 2001): ME (MJ/kg) = 53 + 38 × [CP (%) + 2.25 × ether extract (%) + 1.1 × starch (%) + sugar (%)]. Both diets were presented similarly in pelleted form to reduce differences in feed physical form, to ensure the same quality and to prevent feed selection by hens (Buchanan and Moritz, 2009). Feed and water were provided ad libitum throughout the entire trial. Hen mortality was recorded as it occurred.

Preparation of Feed Ingredients

The 2 dietary protein sources evaluated were SBM (44% CP) and SFM (*Heliantus annus* L., 36% CP and 17.4% crude fiber on DM basis). The major grain energy source for both diets, wheat middlings, was obtained from durum wheat (*Triticum durum* Desf. cv. Appulo). The wheat middlings were previously sieved to separate the fibrous components to obtain a product with average crude fiber content ~3% (Laudadio et al., 2011). The SFM, from commercial supplier, was used as a starting research material. Sunflower meal was micronized in a KMX-300 device (100–200 kg/h; Separ Microsystem, Brescia, Italy), which consists of a steel drum containing a rotor operating at a peripheral speed of approximately 175 m/s. Particle size reduction is accomplished by mechanical impact against stator and rotor serrated surfaces and by turbulent collision between particles. The turbulence and large air volume ensure minimal temperature increase and guarantee a continuous discharge of fine powder through a screen less drain pipe to a cyclone separator and a collector. The meal fractionation was carried out through a turbo-separator (a highly modified cyclone) and a cyclone, which were assembled in series. The apparatus sorted meal into 2 portions: coarse and fine fractions, respectively (Laudadio et al., 2013). The obtained meal was fractioned by air classification with an SX-100 apparatus (Separ Microsystem, Brescia, Italy). For this trial we used the SFM fine fraction highest in protein and

Table 1. Ingredients and chemical analysis of experimental diets fed to laying hens

Item	Diet	
	Soybean meal	Sunflower meal
Ingredient, g/kg (as-fed basis)		
Wheat middlings[1]	706.2	697.8
Soybean meal (44% CP)	153.0	—
Sunflower meal (42% CP)[2]	—	160.0
Calcium carbonate	93.0	93.0
Sunflower oil	20.0	20.0
Dicalcium phosphate	14.5	14.5
Vitamin-mineral premix[3]	2.5	2.5
L-Lys HCl	2.5	4.5
DL-Met	2.2	1.6
Sodium bicarbonate	1.8	1.8
L-Thr	1.8	1.8
Sodium chloride	1.5	1.5
Enzyme[4]	0.5	0.5
Choline chloride	0.5	0.5
Chemical analysis, %		
DM	90.2	90.5
CP	17.4	17.5
Crude fiber	3.7	3.6
Crude fat	4.7	4.5
Ash	13.0	13.3
Calculated analysis		
ME, kcal/kg of diet	2,675	2,681
Lys, %	0.81	0.81
Ca, %	3.91	3.90
Na, %	0.16	0.16
Met + Cys, %	0.74	0.74
Available P, %	0.37	0.37

[1]Low-fiber wheat middlings obtained from durum wheat (Laudadio and Tufarelli, 2011).

[2]Low-fiber sunflower meal obtained from micronized and air-classified sunflower meal.

[3]Provided per kilogram of diet: 6,250 IU of vitamin A; 750 IU of vitamin D_3; 18.75 μg of 25-hydroxycholecalciferol; 15 mg of vitamin E; 1.25 mg of vitamin K_3; 10 mg of niacin; 0.75 mg of vitamin B_1; 2.5 mg vitamin B_2; 5 mg of D-pantothenic acid; 1 mg of vitamin B_6; 0.015 mg of vitamin B_{12}; 0.38 mg of folic acid; 0.05 mg of D-biotin; 25 mg of Fe; 2.5 mg of Cu; 75 mg of Mn; 0.10 mg of Co; 37.5 mg of Zn; 0.50 mg of I; 0.05 mg of Se.

[4]Provided per kilogram of diet: endo-1,4-β-glucanase, 400 U; endo-1,3(4)-β-glucanase, 900 U; endo-1,4-β-xylanase, 1,300 U.

Table 2. Chemical composition of raw sunflower meal and treated sunflower meal

Nutrient, % (as-fed basis)	Raw sunflower meal	Treated sunflower meal[1]
DM	89.7	89.8
CP	36.1	42.3
Ether extract	2.4	2.9
Crude fiber	17.4	10.3
NDF[2]	34.1	16.8
ADF[2]	25.7	13.0
Lignin	7.6	3.8
Ash	6.3	6.8
Acid insoluble ash	0.4	0.3
Hemicellulose	8.4	3.7
Cellulose	18.1	9.6

[1]Low-fiber sunflower (*Helianthus annus* L.) meal obtained after sieving and air classification processes.

[2]NDF = neutral detergent fiber; ADF = acid detergent fiber.

lowest in fiber contents as reported in our previous paper (Laudadio et al., 2013). The chemical composition of the SFM before and after the processing treatments is shown in Table 2.

Sample Collection and Procedures

Samples of diets and raw and treated SFM were ground in a hammer mill with a 1-mm screen and analyzed in triplicate for DM (945.15), ash (967.05), CP (Kjeldahl N × 6.25, 990.03), crude fiber (978.10), and ether extract (945.16) according to AOAC International (2004). The NDF (using heat-resistant α-amylase without sodium sulfite), ADF, and lignin were analyzed according to Mertens (2002), AOAC International (2004; 973.187), and Van Soest et al. (1991), respectively, using the sequential procedure and the filter bag system (Ankom Technology, New York, NY). The NDF and ADF fractions include residual ash.

Body weight of hens was recorded at the start and end of the experiment. Eggs were collected daily and egg production was calculated on a hen-day basis. Eggs with any adhering manure were classed as dirty, and the percentage calculated. Feed intake was recorded weekly by replicate. Feed conversion ratio was calculated as grams of feed per gram of egg. Eggs produced the last day of each week of trial were individually weighed and graded as described by the European Council Directive (2006). The categories recorded for egg size were extra large (>73 g), large (73–63 g), medium (63–53 g), and small (<53 g). Eggs were analyzed for their interior and exterior quality as reported by Laudadio and Tufarelli (2010). Eggs were examined for shell quality by specific gravity. Shell thickness (with shell membrane) of the eggs (30% of the daily egg produced) was measured by micrometer. Shell thickness was a mean value of measurements at 3 locations on the eggs (air cell, equator, and sharp end). Breaking strength of uncracked eggs was determined with a testing machine (model 1140, Instron Ltd., Bucks, UK). Egg components (as albumen, yolk, and shell) were measured by weekly breakouts on 2 eggs per replicate pen and expressed as percentage of egg weight. The Haugh unit was calculated as Haugh units (%) = $100 \times \log (H + 7.57 - 1.7 W^{0.37})$, where H is the height of the albumen and W is the weight of the egg, according the formula proposed by Haugh (1937). Egg yolk color was scored using the 15-point scale of the DSM yolk color fan (DSM Nutritional Products Ltd., Basel, Switzerland).

Blood samples (2.0 mL) from 20% of hens in each replicate (n = 5) were collected weekly during the whole feeding period. Blood was collected from the brachial wing vein using sterilized syringes and needles. After 1 h standing at room temperature, serum was isolated by centrifugation at 1,150 × *g* for 10 min. Serum samples were stored at −80°C until further analysis.

Yolk and Serum Cholesterol Contents

The yolk cholesterol and high-density lipoprotein (**HDL**) cholesterol concentrations were weekly determined in egg yolk (1 g) saponified with 20 mL of 33% ethanolic KOH in tightly capped tubes placed in a 60°C water bath for 1 h. The mixture was then cooled in ice water, and 5 mL of distilled water was added. Cholesterol in unsaponifiable fractions was extracted twice with 5 mL of hexane. The resulting aliquot of hexane containing cholesterol was dried under nitrogen, redissolved in 5 mL of hexane, and injected into a gas chromatograph (HP-6890 N, Agilent, Palo Alto, CA). Five α-cholestane (Sigma-Aldrich) was used as an internal standard. A split inlet (split ratio, 100:1) was used to inject samples into a capillary column (HP-5, Agilent, Santa Clara, CA; 30 m × 0.53 mm × 0.5 μm), and the gradually increased oven temperature was 270°C isothermal, detector temperature was 300°C, and inlet temperature was 210°C. The N_2 served as the carrier gas at a constant flow rate of 1.0 mL/min. Total cholesterol concentrations in the plasma was analyzed independently by UV spectrophotometer using commercial kits (Diasys, Diagnostic Systems, Holzheim, Germany). Low-density lipoprotein (**LDL**) cholesterol was estimated using the Friedewald et al. (1972) equation. The atherogenic index was calculated as the ratio of LDL cholesterol to HDL cholesterol. Cholesterol and HDL cholesterol concentrations in serum were determined using the same reagent kits as those used for yolk analysis.

Statistical Analysis

Data were analyzed using the 1-way ANOVA option of the GLM of SAS/STAT software (SAS Institute Inc., 2004) as a completely randomized design with the 2 dietary treatment or CP sources as main effects. The experimental unit was the pen. The statistical model used was

$$Y_{ijk} = \mu + P_i + \varepsilon_{ijk},$$

Table 3. Effect of the diets on laying hens' growth performance, egg production, and weight[1]

	Diet			
Item	Soybean meal	Sunflower meal	SEM	*P*-value
Initial BW, g	1,817	1,820	6.31	0.544
Final BW, g	1,879	1,885	8.71	0.413
ADFI, g	122.5	121.7	0.27	0.077
FCR, g of feed/g of egg	1.96	1.92	0.05	0.059
Egg-laying rate, %	92.7	93.2	0.44	0.085
Egg weight, g	62.6	63.5	0.39	0.052
Mortality, %	1.2	1.1	—	0.698

[1]Values are means of 4 observations. FCR = feed conversion ratio.

where Y_{ijk} = response variables from each individual replication or pen, μ = the overall mean, P_i = the effect of dietary protein source, and ε_{ijk} = the intraexperimental unit error term. Means were compared by the Student-Newman-Keul's method. Significance indicates $P < 0.05$ unless stated otherwise.

RESULTS AND DISCUSSION

The proximate chemical composition of raw and treated SFM is reported in Table 2. The combination of sieving and air classification of SFM was effective in separating protein and fiber from the starting material. Low-fiber SFM was found to contain appreciable content of nutrients; in fact, the sieving and air classification processes improved CP (361 vs. 423 g/kg, as-fed basis) and ether extract (24 vs. 29 g/kg) levels and reduced crude fiber (174 vs. 103 g/kg) and neutral detergent fiber (341 vs. 168 g/kg) compared with untreated meal. Sieving and air classification are used to produce protein or concentrates mainly in cereals and pulses (Srinivasan et al., 2012; Laudadio et al., 2013). Meal particles obtained differ in their shape, size, and density. Air classification differentiates the protein (fine fraction) and starch (coarse fraction) particles (Owusu-Ansah and McCurdy, 1991; Zhou et al., 2013).

Based on our findings, low-fiber SFM has shown the valuable potential for commercial application of this technology, and the fractionation of nutritional components from sunflower can be also efficiently done by sieving and air classification. The significant protein content of low-fiber SFM obtained in our trial has nutritional significance because moderate intake of this meal will greatly increase the total dietary protein intake. Hence, its utilization as an alternative protein source in poultry formulation will reduce the overdependence on conventional plant protein such as SBM. A substantial amount of fiber is needed for normal digestive function, but high-fiber ingredients are avoided in poultry diets mainly because of their low energy values. The acceptable range of dietary crude fiber is 35 to 45 g/kg in a practical laying hen diet (Shi et al., 2012). The crude fiber of SFM, depending on the extent of dehulling, appears to be the most crucial aspect in poultry diets (Senkoylu and Dale, 2006). In fact, the high level of fiber is always associated with slow passage rate for feed in the digestive system, which may depress performance of the birds (Connell, 1981). Table 3 shows the effects of a diet including low-fiber meal on BW, feed consumption, FCR, egg production, and egg weight of laying hens. Including 160 g/kg of low-fiber SFM as total substitute of soybean in the laying hen diet did not affect ($P > 0.05$) BW, feed consumption, or FCR during the 10-wk feeding trial.

Concerning the effects of dietary SFM on BW changes, feed intake of layers, and feed efficiency, the available results are questionable and contrasting. In a previous study, Vieira et al. (1992) reported that dietary SFM up to 15% increased feed consumption of laying hens, but FCR results negatively correlated with SFM level. Moreover, in accordance with our findings, Casartelli et al. (2006) and Shi et al. (2012) reported no statistical difference among SFM inclusion levels of for layer performance parameters. However, other studies showed that addition of SFM into the hen diet significantly affected BW and feed intake of hens (Furlan et al., 2001). Because of the increase in body mass of layers was negatively correlated with egg production, the stability of body mass in hens fed diets supplemented with different protein sources can be considered a favorable factor in increasing egg production (Laudadio and Tufarelli, 2010). The results of the present study showed that total substitution of SBM with low-fiber SFM in the diet did not negatively influence hen weight changes and then dietary low-fiber SFM led to better trends in feed utilization. The determination of FCR done by considering total egg production and total feed intake is perhaps the major single index used in economic assessment of egg production in laying hens (Laudadio and Tufarelli, 2011).

The egg-laying rates (%) were similar ($P = 0.085$) for all hens fed the experimental diets with a range of 92.7 in hens on soybean diet and 93.2 in hens on low-fiber sunflower diet. Further, the egg weight was also statistically similar ($P = 0.052$) among experimental hens, with a range 62.6 and 63.5 g for hens fed SBM or low-fiber SFM diet, respectively. The average weight of the eggs also conformed and compared favorably with values reported for layers in available literature (Alshami et al., 2011; Shi et al., 2012). The only significant difference observed of the egg traits was for the percentage medium-size eggs (53–63 g) from 28 to 38 wk of

Table 4. Effects of diets on the egg-quality parameters, egg yolk, and serum cholesterol in laying hens[1]

	Diet			
Item	Soybean meal	Sunflower meal	SEM	*P*-value
Egg grade, %				
>73 g	3.9	4.4	0.05	0.112
63 to 73 g	47.0	48.1	0.39	0.032
53 to 63 g	42.2	41.2	0.31	0.039
<53 g	6.9	6.3	0.06	0.213
Haugh unit	88.01	89.42	0.41	0.089
Shell thickness, mm $\times 10^{-2}$	0.32	0.34	<0.01	0.401
Shell strength, kg/cm^2	1.55	1.57	0.03	0.337
Broken + shell-less eggs, %	0.17	0.16	<0.01	0.455
Dirty eggs, %	0.32	0.31	<0.01	0.476
Egg components, %				
Yolk	24.9	25.0	0.14	0.053
Albumen	64.6	64.2	0.12	0.066
Shell	10.5	10.8	0.05	0.202
Yolk color score	10.97	12.01	0.17	0.027
Yolk				
Cholesterol, mg/g of yolk	12.91	11.05	0.44	0.009
HDL cholesterol,[2] mg/g of yolk	6.21	7.44	0.32	0.011
LDL cholesterol,[2] mg/g of yolk	4.15	3.77	0.25	0.032
Atherogenic index[2]	0.67	0.53	0.07	0.029
Serum				
Cholesterol, mg/dL	120.1	102.3	7.69	0.006
HDL cholesterol, mg/dL	67.9	71.1	2.53	0.009
LDL cholesterol,[2] mg/dL	101.9	86.7	1.21	0.004
Atherogenic index[3]	1.5	1.2	0.31	0.017

[1]Values are means of 4 observations.

[2]HDL = high-density lipoprotein. Low-density lipoprotein (LDL) cholesterol = (total cholesterol − HDL cholesterol) – triglycerides/5 (Friedewald et al., 1972).

[3]Atherogenic index: the ratio of LDL cholesterol to HDL cholesterol (LDL/HDL).

age that was greater for hens fed the SBM control diet than for hens fed low-fiber sunflower (42.2 vs. 34.5%; $P = 0.047$) and large-size eggs (63–73 g) production that was higher for hens fed low-fiber SFM ($P = 0.047$; Table 4). Dietary treatment did not negatively affect any trait related to egg or shell quality (Table 4). The overall values obtained in the present study are quite acceptable for optimal egg quality for this age of hens (28 to 38 wk) in the phase of production. Conversely, previous studies on hens have reported that sunflower supplementation can have a negative effect on egg quality (Karunajeewa et al., 1989). However, this may be related to the presence of the high fiber content in the SFM, in our case minimized by the processing effect of sieving and air classification. In fact, a combination of processing effects has been reported to be effective in reducing the levels of crude fiber also in other feed ingredients (Srinivasan and Singh, 2008; Challa et al., 2010; Laudadio et al., 2013). The shell thickness and strength also had similar mean values ($P = 0.355$) among dietary treatments for each parameter, indicating a similar relative density for the eggs. Moreover, the Haugh unit values were also similar ($P = 0.119$) for the eggs laid by the experimental laying birds. The average values of our findings on egg-quality parameters conformed to data reported in the available literature (Tsuzuki et al., 2003; Shi et al., 2012). The egg yolk color score was increased when low-fiber SFM was included in the diet of layers compared with the group fed SBM (12.01 vs. 10.97, respectively; $P = 0.027$). The influence of low-fiber SFM on yolk color observed in our study could be related to the quantity of natural pigments contained in the alternative ingredient. Previous studies (Güçlu et al., 2004; Laudadio and Tufarelli, 2010) showed a progressive effect in yolk color as level of leguminous plants in laying hen diet was increased.

Table 4 shows the effect of low-fiber SFM supplementation on egg yolk and serum cholesterol. Egg yolk cholesterol was decreased significantly by inclusion of low-fiber SFM in the laying hens diet compared with those fed soybean-control diet (11.05 vs. 12.91 mg/g yolk; $P = 0.009$). The same was also true for serum cholesterol concentrations between treatments ($P = 0.006$). The egg yolk and serum HDL cholesterol increased significantly for laying hens fed diet supplemented with low-fiber SFM compared with the control treatment. The atherogenic index and HDL cholesterol resulted significantly lower in the serum and egg yolk of the group fed the diet including low-fiber SFM compared with the other dietary group. According to the current study, a significant decrease was found in egg yolk cholesterol of hens when soybean was replaced with different levels of SFM (Shi et al., 2012). The hypocholesterolemic effect of low-fiber SFM in serum and egg yolk should be partly due to suppression of de novo lipogenesis in the liver. However, it is unknown whether SFM supplementation is effective in reducing intestinal reabsorption of biliary cholesterol in laying hens, which modulates

whole-body cholesterol in favor of lowering plasma and yolk cholesterol content. Moreover, reduced yolk cholesterol content as a result of feeding low-fiber sunflower could be partly due to the presence of plant sterols in this feed ingredient, which have a hypocholesterolemic effect (Liu et al., 2010).

The substitution of SBM with low-fiber SFM in layer diets under the present study did not cause any adverse effects on egg production and egg quality compared with a conventional diet. These findings indicated that dietary low-fiber SFM is in a suitable protein source to support laying performance and improve egg quality as well as to develop low-cholesterol eggs.

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