Low-fiber alfalfa (*Medicago sativa* L.) meal in the laying hen diet: Effects on productive traits and egg quality

V. Laudadio,* E. Ceci,† N. M. B. Lastella,* M. Introna,* and V. Tufarelli*[1]

*Department of Emergency and Organ Transplantation (DETO), Section of Veterinary Science and Animal Production, and †Department of Veterinary Medicine, University of Bari "Aldo Moro," Valenzano 70010, Italy

ABSTRACT This study was designed to determine the effects on laying performance and egg quality resulting from partial substitution of soybean meal (SBM) with low-fiber alfalfa (LFA; *Medicago sativa* L.) meal in the diet of early-phase laying hens. ISA Brown layers, 18 wk of age, were randomly allocated to 2 dietary treatments and fed for 10 wk. The hens were fed 2 wheat middling–based diets: a control diet, which contained SBM (15% of diet), and a test diet containing LFA (15% of diet) as the main protein source. Low-fiber alfalfa meal was obtained by a combination of sieving and air-classification processes. Feed intake was recorded daily, and egg production was calculated on a hen-day basis; eggs from each group were weekly collected to evaluate egg components and quality. The partial substitution of SBM with LFA had no adverse effect on growth performance of early-phase laying hens. Egg production and none of the egg-quality traits examined were influenced by dietary treatment, except for yolk color ($P < 0.001$) and yolk percentage ($P < 0.05$) as well as yolk cholesterol and β-carotene contents ($P < 0.001$), which were improved in hens fed the LFA diet. Including LFA increased serum β-carotene and reduced serum cholesterol concentrations ($P < 0.001$). Our results suggest that partially replacing conventional SBM as protein source with low-fiber alfalfa meal in the laying-hen diet can positively influence yolk quality without adversely affecting productive traits.

Key words: alfalfa meal, laying hen, egg quality, cholesterol, β-carotene

2014 Poultry Science 93:1868–1874
http://dx.doi.org/10.3382/ps.2013-03831

INTRODUCTION

In poultry feeding, soybean meal (**SBM**) is usually the main protein source because it has a high protein content, has a balanced amino acid profile, and is a satisfactory source of essential fatty acids (Hammershøj and Steenfeldt, 2005). An increase in world SBM demand due to the increase in poultry production, associated with a stabilization of SBM production, has led to a decrease in availability and an increase in price of this commodity (Laudadio et al., 2011).

Alfalfa (*Medicago sativa* L.) meal is a commercially available feedstuff moderately rich in protein but with high concentrations of fiber. Alfalfa is well balanced in amino acids and is a rich source of minerals as well as vitamins (Jiang et al., 2012). Xanthophylls, β-carotene, and flavonoids in alfalfa are responsible for the high antioxidant properties of this plant (Aziz et al., 2005). In particular β-carotene is an important bioactive substance in alfalfa that is a precursor of vitamin A and retinoid, which has been defined as an important molecule in animal nutrition (Schweigert et al., 2002). Such a broad range of effects makes alfalfa meal an excellent poultry feed ingredient with different purposes (Khan et al., 2011). Recent studies showed alfalfa could reduce the concentrations of cholesterol in the meat and egg yolk (Dong et al., 2007; Krauze and Grela, 2010). Güçlu et al. (2004) reported that adding up to 9% alfalfa meal to the diet of laying quails had no significant effect on live weight, egg production, feed intake, and efficiency. Nevertheless, the use of alfalfa in poultry feeding is limited due to the high fiber content. This problem could be overcome by reducing the fiber level of alfalfa, 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; Laudadio and Tufarelli, 2010). Combining processes can be used to improve the enrichment of fractions. 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; Srini-

Received December 13, 2013.
Accepted March 26, 2014.
[1]Corresponding author: vincenzo.tufarelli@uniba.it

vasan et al., 2012). However, in spite of these successes, to the best of our knowledge, the application of sieving air classification to alfalfa meal was never previously investigated. Therefore, the aim of this study was to evaluate the inclusion of low-fiber alfalfa meal as alternative protein sources 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 facility located at the University of Bari "Aldo Moro," Italy, observing the animal welfare Legislative Decree 116/92, Council Directive 98/58/EC, received in Italy by Legislative Decree 146/2001, and Council Directive 2007/43/CE, received in Italy by the governmental Decree 181/2010 and Legislative Decree 267/2003.

A total of 200 ISA Brown laying hens, 18 wk of age with an initial BW of 1,571 ± 12.2 g, were free-range reared. The hens were divided into 2 groups of 100 hens each (4 replicates each of 25 birds per 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).

Dietary Treatments

The hens were fed 2 experimental diets: a wheat middling-soy diet (control) and wheat middling-alfalfa-soy diet. Each diet was replicated 4 times, with each replicate comprising one pen of 25 birds. The diets (Table 1) were isocaloric and isonitrogenous containing 17.5% CP and 2,680 kcal of ME/kg of diet, designed to meet or exceed the nutrient requirements for early-phase laying hens (NRC, 1994). The diets were supplemented with a commercial feed enzyme (Roxazyme G2G; Roche, Basel, Switzerland). 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 (48% CP) and alfalfa (*Medicago sativa* L., 19.2% CP and 26.7% crude fiber on a 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 an average crude fiber content of ~3% (Laudadio et al., 2011).

Alfalfa meal, organically and locally grown, was used as a starting research material. The fractionation was carried out through a turbo-separator (a highly modified cyclone) and a cyclone that were assembled in series. The apparatus sorted meal into 2 portions: coarse and fine fractions (Laudadio et al., 2013). The obtained meal was fractioned by air classification with an SX-100 apparatus (Separ Microsystem, Brescia, Italy). Briefly, this apparatus has a working capacity of 100 to 200 kg/h and consists of a turbo-separator (a highly modified cyclone) and a cyclone assembled in series. An aspirating pump mounted at the end of the system drives the air flow, which is modulated by means of an inlet valve. A further cyclone and a filter are placed before the pump to remove any very fine powder, which never exceeded 1% of the initial meal and is thus not discussed at any point (Ferrari et al., 2009). The apparatus sorted the flour into 2 portions (coarse and

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

Item	Soybean meal	Alfalfa meal
Ingredient, g/kg, as-fed basis		
Wheat middlings[1]	705.6	626.7
Soybean meal (48% CP)	150.0	80.0
Alfalfa meal (26% CP)	—	150.0
Calcium carbonate	90.0	86.0
Sunflower oil	20.0	26.0
Dicalcium phosphate	17.0	17.5
Vitamin-mineral premix[2]	5.0	2.5
L-Lys HCl	3.2	2.6
Sodium chloride	2.5	1.6
Sodium bicarbonate	2.4	2.2
DL-Met	1.3	2.8
Thr	1.0	1.0
Enzyme[3]	1.0	1.0
Choline chloride	1.0	0.5
Chemical analysis, %		
DM	90.2	89.8
CP	17.5	17.5
Crude fiber	3.8	3.9
Crude fat	4.9	5.0
Ash	12.8	12.7
Calculated analysis		
ME, kcal/kg of diet	2,675	2,689
Lys, %	0.80	0.81
Ca, %	3.91	3.94
Met + Cys, %	0.74	0.74
Available P, %	0.62	0.62
Fatty acid,[4] %		
Σ SFA	30.93	29.42
Σ MUFA	34.54	35.21
Σ PUFA	34.53	35.37
Total n-6	32.61	34.17
Total n-3	1.57	2.11

[1]Wheat middlings obtained from durum wheat (*Triticum durum* Desf. cv. Appulo; Laudadio and Tufarelli, 2011).

[2]Provided per kilogram of product: 2,500,000 IU of vitamin A; 300,000 IU of vitamin D_3; 7,500 μg of 25-hydroxycholecalciferol; 6,000 mg of vitamin E; 500 mg of vitamin K_3; 4,000 mg of niacin; 300 mg of vitamin B_1; 1,000 mg of vitamin B_2; 2,000 mg of D-pantothenic acid; 400 mg of vitamin B_6; 3 mg of vitamin B_{12}; 150 mg of folic acid; 20 mg of D-biotin; 10,000 mg of Fe; 1,000 of mg Cu; 30,000 mg of Mn; 40 mg of Co; 15,000 mg of Zn; 200 mg f I; 20 mg of Se. Assayed concentrations of β-carotene for the 2 diets were 0.31 and 0.89 mg/kg, respectively.

[3]Provided per kilogram of product: endo-1,4-β-glucanase 800,000 U; endo-1,3(4)-β-glucanase 1,800,000 U; endo-1,4- β-xylanase 2,600,000 U.

[4]SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid.

fine fractions), which were collected from the turboseparator and the cyclone, respectively. The air-flow inlet restriction valve was the only adjustable setting of the apparatus. The relative yield of the coarse and fine fractions could be changed in a consistent way by varying the air flow. Because the set value of the airflow valve is of significance only for the apparatus used here, the yield of the coarse fraction was assumed as a more correct reference parameter for the air-classification process. For this trial we used the alfalfa fine fraction highest in protein and lowest in fiber contents. The chemical composition of the alfalfa meal before and after the processing treatments is shown in Table 1.

Sample Collection and Procedures

Samples of diets and raw and treated alfalfa meal 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 (2000). The neutral detergent fiber (using heat-resistant α-amylase without sodium sulfite), acid detergent fiber, and lignin were analyzed according to Mertens (2002), AOAC International (2000) (973.187), and Van Soest et al. (1991), respectively, using the sequential procedure and the filter bag system (Ankom Technology, New York, NY).

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 was calculated. Feed intake was recorded weekly by replicate. Feed conversion ratio was calculated as grams of feed per grams of egg. Eggs produced the last day of each week on 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) as reported by Safaa et al. (2009). 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 (10% 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 calculates as Haugh units (%) $= 100 \times \log (H + 7.57 - 1.7W^{0.37})$, where H is the height of the albumen and W is the weight of the egg, according the formula proposed by Card and Nesheim (1972). Yolk color was determined by the Roche yolk color fan (Hoffman-La Roche Ltd., Basel, Switzerland; color scale from 15, dark orange, to 1, light pale).

Blood samples (2.0 mL) from each individual layer 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 at 20°C. Serum samples were stored at −80°C until further analysis.

Yolk and Serum Cholesterol and β-Carotene Contents

The yolk cholesterol concentrations were determined sampling egg yolks (1 g) weekly (using the 20% of the eggs produced in a week) 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, Hewlett-Packard, Palo Alto, CA). Five α-cholestane (Sigma-Aldrich, Oakville, Canada) 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 carrier gas was N_2 at a constant flow rate of 1.0 mL/min. Total cholesterol concentrations in the plasma were analyzed independently by UV spectrophotometer using commercial kits (DiaSys Diagnostic Systems, Holzheim, Germany).

Concentrations of yolk and serum β-carotene were measured using HPLC according to the method outlined by Cucco et al. (2007) and Mori et al. (2003), respectively. Briefly, an aliquot of yolk (0.2–0.5 g) was homogenized in 2 mL of a 1:1 (vol/vol) mixture of 5% NaCl solution and ethanol, followed by the addition of 3 mL of hexane and further homogenization for 3 min. After centrifugation, hexane was collected and the extraction was repeated twice. Hexane extracts were combined and evaporated under N_2, the residue was dissolved in 1 mL of methanol:dichloromethane (1:1, vol/vol) and centrifuged, and the supernatant was used for carotenoid determination. The β-carotene was determined by HPLC with a WatersTM Alliance 2695 Modul-System (Millipore Corp., Bedford, MA), using a Spherisorb type S3ODS2, 5-m C18, reverse-phase column, 25 mm (Phase Separation, Clwyd, UK) with a mobile phase of acetonitrile–methanol (85:15) and acetonitrile–dichloromethane–methanol (70:20:10) in gradient elution using detection by absorbance at 445 nm. Peaks were identified by comparison with the retention

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

Nutrient, %, as-fed basis	Raw alfalfa meal	Treated alfalfa meal[1]
DM	89.4	89.6
CP	19.2	26.3
Ether extract	2.1	2.8
Crude fiber	26.7	15.3
Neutral detergent fiber	45.2	28.5
Acid detergent fiber	34.3	23.0
Lignin	9.5	5.8
Ash	10.3	11.3
Acid insoluble ash	0.09	0.06
Hemicellulose	10.9	5.5
Cellulose	23.8	16.6

[1]Low-fiber alfalfa (*Medicago sativa* L.) meal obtained after sieving and air-classification processes.

times of carotenoid standards and integrated with specific software.

Statistical Analysis

Data were analyzed using the one-way ANOVA option of the GLM of SAS/STAT software (SAS Institute Inc., 2000) as a completely randomized design with the 2 dietary treatment or CP sources (SBM and LFA) as main effects. The statistical model used was $Y_{ijk} = \mu + P_i + R_{ij} + \varepsilon_{ijk}$, where Y_{ijk} = response variables from each individual replication or pen; μ = the overall mean; P_i = the effect of dietary protein source; R_{ij} = the interexperimental unit (replications) error term; and ε_{ijk} = the intraexperimental unit error term. When there was a significant F-value, means were compared by the Student-Newman-Keul's method. Significance implies $P < 0.05$ unless stated otherwise.

RESULTS AND DISCUSSION

No literature was located on the effects of sieving and air-classification processes on the nutritional properties of alfalfa meal and its effect on laying hens productive performance. Thus, this subject should be considered as a new investigation.

The proximate composition of raw and treated alfalfa meal is reported in Table 2. The combination of sieving and air classification of alfalfa meal was effective in separating protein and fiber from starting material. Low-fiber alfalfa meal was found to contain appreciable content of nutrients; in fact the sieving and air-classification processes improved CP (192 vs. 263 g/kg as-fed basis) and ether extract (21 vs. 28 g/kg) level and reduced crude fiber (267 vs. 153 g/kg) and neutral detergent fiber (452 vs. 285 g/kg) level compared with untreated meal.

Sieving and air classification are used to produce protein or concentrates mainly in cereals and pulses (Srinivasan et al., 2012). Meal particles produced are different in their shape, size, and density. Air classification differentiates the protein (fine fraction) and starch (coarse fraction) particles (Owusu-Ansah and McCurdy, 1991). The meal is air classified in a spiral air stream and fractionated into light and heavy particles. The fine and light particles contain protein, whereas the coarse and heavier particles mostly contain starch granules. Based on our findings, low-fiber alfalfa meal has shown the valuable potential for commercial application of this technology, and the fractionation of nutritional components from alfalfa can be also efficiently done by sieving and air classification. The notable protein content of low-fiber alfalfa 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 over-dependence on conventional plant protein such as soybean meal. Moreover, *Medicago sativa* is rich in Try, Lys, and Thr; therefore, the obtained production results may be used to prove high efficiency of protein alfalfa meal application as poultry feed (Krauze and Grela, 2010).

In Table 3 are reported the effects of a diet including low-fiber meal on the BW, feed consumption, feed conversion ratio, egg production, and egg weight of early-phase laying hens. A diet containing alfalfa had no significant effects on hens final BW ($P = 0.596$). Inclusion of the alternative protein source at our level of 15% in the diet had no significant ($P = 0.058$) influence on feed intake, without any negative effect also on feed efficiency ($P = 0.092$) compared with the control diet containing conventional soybean meal.

Concerning the effects of dietary alfalfa on BW changes, feed intake of layers, and feed efficiency, the data results are questionable and contrasting. In a previous study, Güçlu et al. (2004) reported that dietary alfalfa meal at different inclusion levels had no significant effect in feed consumption of laying hens, but feed

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

Item	Soybean meal	Alfalfa meal	SEM	P-value
Initial BW, g	1,565	1,582	8.74	0.752
Final BW, g	1,802	1,825	9.89	0.596
ADFI, g	117.1	118.3	0.31	0.058
Feed conversion ratio, g of feed/g of egg	1.92	1.94	0.03	0.092
Egg-laying rate, %	88.79	89.01	0.51	0.177
Egg weight, g	61.4	62.2	0.32	0.059

Table 4. Effects of diets on the egg-quality parameters and serum cholesterol and β-carotene

Item	Soybean meal	Alfalfa meal	SEM	*P*-value
Egg grade, %				
>73 g	4.0	3.5	0.07	0.202
63–73 g	58.5	60.5	0.46	0.041
53–63 g	36.5	34.8	0.33	0.047
<53 g	1.0	1.2	0.04	0.336
Haugh unit	87.05	89.71	0.45	0.091
Shell thickness, mm × 10^{-2}	0.31	0.33	<0.01	0.355
Shell strength, kg/cm^2	1.52	1.59	0.03	0.253
Broken + shell-less eggs, %	0.16	0.15	<0.01	0.522
Dirty eggs, %	0.32	0.29	<0.01	0.203
Egg components, %				
Yolk	23.7	25.4	0.11	0.037
Albumen	65.5	64.1	0.15	0.051
Shell	10.8	10.5	0.06	0.144
Yolk color score	11.14	13.27	0.23	<0.001
Yolk				
Cholesterol, mg/g of yolk	13.1	10.2	0.50	<0.001
β-Carotene, μg/g of yolk	153.2	302.5	20.21	<0.001
Serum				
Cholesterol, mg/dL	117.5	101.1	8.22	<0.001
β-Carotene, μg/mL	32.4	120.7	10.53	<0.001

conversion ratio results were negatively correlated with alfalfa meal level. However, other studies showed that addition of alfalfa meal into the hen diet significantly affected BW and intakes of the hens (Güçlu et al., 2004). Because the increase in body mass of layers was negatively correlated with egg production, the stability of body mass in hens fed diets supplemented with lupin can be considered a favorable factor in increasing egg production as reported by Aydin et al. (2008) and more recently by Laudadio and Tufarelli (2010). The findings of the present study showed that partial substitution of soybean meal with low-fiber alfalfa meal in the diet did not negatively influence hen weight changes and then dietary alfalfa led to better feed utilization. Such aspects include nutrient content, house temperature, rate of production, egg size, and BW. The suggested feed intake rate for the early-phase ISA Brown strain hens under normal field conditions using an energy adequate diet was also met. The determination of feed conversion ratio done by considering the total egg production and total feed intake is perhaps the major singular index used in economic assessment of egg production in laying hens (Laudadio and Tufarelli, 2010).

The egg-laying rates (%) were statistically similar ($P = 0.177$) for all hens on experimental diets with a range of 88.8 in hens on soybean diet and 89.0 in hens on low-fiber alfalfa diet, indicating uniformity in the laying pattern and quantity of egg laid by the hens fed alternative protein source. It is suggestive that with dietary inclusion of treated alfalfa meal, there was the possibility of an increased blood flow to the ovaries, thereby leading to more ovarian follicle formation, which ultimately increased the egg production (Fasuyi et al., 2007). The egg weight was also statistically similar ($P = 0.059$) among experimental hens, with a range 61.4 and 62.2 g for hens fed SBM or LFA diet, respectively, indicating that the inclusion of the alternative protein source supported this egg trait as also previously found by Güçlu et al. (2004). The average weight of the eggs also conformed and compared favorably with values reported for layers in available literatures (Khajali et al., 2007; Al-shami et al., 2011). The only significant difference observed of the egg traits was for the percentage of medium-size eggs (53–63 g) from 18 to 28 wk of age that was greater for hens fed the SBM control diet than for hens fed alfalfa (36.5 vs. 34.5%; $P = 0.047$) and large-size eggs (63–67 g) production that resulted higher in hens fed alfalfa meal (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 (18 to 28 wk) in the early phase of production. Conversely, studies in the past, on hens fed diets including alfalfa meal, have reported that alfalfa supplementation can have a negative effect on egg quality (Güçlu et al., 2004). However, this may be related to the presence of the high fiber content in the alfalfa meal, in our case instead 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 ($P = 0.091$) for the eggs laid by the experimental laying birds. The average values of these egg-quality parameters conformed to data reported for standard commercial egg production guides and other available literature (Farran et al., 2001; Laudadio and Tufarelli, 2011). The egg yolk color score was increased when

low-fiber alfalfa meal was included into the diet of layers compared with the group fed SBM (13.27 vs. 11.14, respectively; $P < 0.001$). The influence of LFA on yolk color observed in our study may be related to the quantity of natural pigments contained in the alternative feed 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.

Serum and egg-yolk β-carotene increased for hens given diets supplemented with low-fiber alfalfa meal (Table 4). The same was true for serum and egg-yolk cholesterol concentrations between treatments. The concentrations of β-carotene in the egg yolk were within the ranges reported in other avian species (Surai, 2002). It is well known that enrichment of poultry diets with carotenoids results in increased concentration in the egg yolk. The β-carotene-rich vegetables such as alfalfa meal have been studied to obtain the darkest egg yolk color and stable eggs resulting from their increased antioxidant ability against lipid peroxidation (Cucco et al., 2007). In agreement with the present data, other studies in which carotenoids-rich plants were supplemented to poultry diets resulted in increased egg-yolk carotenoids concentrations (Akdemir et al., 2012). Moreover, high β-carotene concentration may increase the oxidative stability and provide a source of β-carotene that is useful for human nutrition and health. In addition to affecting the yolk color, β-carotene plays a key role as antioxidants in the healthy development of chick embryos, ensuring a stronger immune response (Dong et al., 2007). Regarding the transmission of β-carotene from serum to eggs, it has been shown that dietary supplementation can increase the yolk carotenoid contents (Surai et al., 2003). Nevertheless, it is clear that the dietary variation in carotenoids is not the only factor that produces individual differences; an important role of breed, age, and season was reported (Cucco et al., 2007). Low-fiber alfalfa-meal supplementation in diet markedly decreased ($P < 0.001$) serum and yolk total cholesterol levels. The hypocholesterolemic effect of low-fiber alfalfa meal in serum and egg should be partly owing to suppression of de novo lipogenesis in the liver. However, it is unknown whether alfalfa meal 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 alfalfa can be partly due to the presence of saponins in this ingredient, which have hypocholesterolemic effects (Mourão et al., 2006).

As a result, low-fiber alfalfa (*Medicago sativa* L.) meal can be included in the commercial early-phase laying hen diet with a significant depression in neither production nor quality of eggs, and the implication of these findings indicated a diet with low-fiber alfalfa dietary inclusion may be the most cost-effective suitable diet for laying performance to improve egg quality; however, further trials are needed to give more reliable conclusions about the effects of treated alfalfa meal on layer performance traits.

REFERENCES

Akdemir, F., C. Orhan, N. Sahin, K. Sahin, and A. Hayirli. 2012. Tomato powder in laying hen diets: Effects on concentrations of yolk carotenoids and lipid peroxidation. Br. Poult. Sci. 53:675–680.

Al-shami, M. A., M. E. Salih, and T. E. Abbas. 2011. Effects of dietary inclusion of alfalfa (*Medicago sativa* L.) leaf meal and Xylan enzyme on laying hens' performance and egg quality. Res. Opin. Anim. Vet. Sci. 2:14–18.

AOAC International. 2000. Official Methods of Analysis. 17th ed. AOAC Int., Gaithersburg, MD.

Aydin, R., M. Karaman, T. Cicek, and H. Yardibi. 2008. Black cumin (*Nigella sativa* L.) supplementation into the diet of the laying hen positively influences egg yield parameters, shell quality, and decreases egg cholesterol. Poult. Sci. 87:2590–2595.

Aziz, N., N. L. Paiva, G. D. May, and R. A. Dixon. 2005. Transcriptome analysis of alfalfa glandular trichomes. Planta 221:28–38.

Buchanan, N. P., and J. S. Moritz. 2009. Main effects and interactions of varying formulation protein, fiber, and moisture on feed manufacture and pellet quality. J. Appl. Poult. Res. 18:274–283.

Card, L. E., and M. C. Nesheim. 1972. Poultry Production. 11th ed. Lea Febiger, Philadelphia, PA.

Challa, R., R. Srinivasan, and F. To. 2010. Fractionation of soybean meal, cottonseed meal and wheat middlings using combination of sieving and air classification. Anim. Feed Sci. Technol. 159:72–78.

Cucco, M., B. Guasco, G. Malacarne, and R. Ottonelli. 2007. Effects of β-carotene on adult immune condition and antibacterial activity in the eggs of the Grey Partridge (*Perdix perdix*). Comp. Biochem. Physiol. A Mol. Integr. Physiol. 147:1038–1046.

Dong, X. F., W. W. Gao, J. M. Tong, H. Q. Jia, R. N. Sa, and Q. Zhang. 2007. Effect of polysavone (alfalfa extract) on abdominal fat deposition and immunity in broiler chickens. Poult. Sci. 86:1955–1959.

European Council Directive. 2006. Chapter II: Grades of eggs. Article 7: Grading of Grade A eggs of Regulation (EC) No 2295/2003. Certain Marketing Standards for Eggs. Comm. Eur. Communities, Brussels, Belgium.

Farran, M. T., P. B. Dakessian, A. H. Darwish, M. G. Uwayjan, H. K. Dbouk, F. T. Sleiman, and V. M. Ashkarian. 2001. Performance of broilers and production and egg quality parameters of laying hens fed 60% raw or treated common vetch (*Vicia sativa*) seeds. Poult. Sci. 80:203–208.

Fasuyi, A. O., F. S. A. Dairo, and O. T. Olujimi. 2007. Protein supplementary quality of vegetable leaf meal (*Amaranthus cruentus*) in the diets of laying hens: Egg laying performance, egg quality and heamatological implications. J. Food Agric. Environ. 5:294–300.

Ferrari, B., F. Finocchiaro, A. M. Stanca, and A. Gianinetti. 2009. Optimization of air classification for the production of β-glucan enriched barley flours. J. Cereal Sci. 50:152–158.

Güçlu, B. K., K. M. Işcan, F. Uyanik, M. Eren, and A. C. Ağca. 2004. Effect of alfalfa meal in diets of laying quails on performance, egg quality and some serum parameters. Arch. Anim. Nutr. 58:255–263.

Hammershøj, M., and S. Steenfeldt. 2005. Effects of blue lupin (*Lupinus angustifolius*) in organic layer diets and supplementation with foraging material on egg production and some egg quality parameters. Poult. Sci. 84:723–733.

Jiang, J. F., X. M. Song, X. Huang, J. L. Wu, W. D. Zhou, H. C. Zheng, and Y. Q. Jiang. 2012. Effects of alfalfa meal on carcass quality and fat metabolism of Muscovy ducks. Br. Poult. Sci. 53:681–688.

Khajali, F., A. K. Zamani Moghaddam, and E. Asadi Khoshouie. 2007. Application of an early skip-a-day feed restriction on physiological parameters, carcass traits and development of ascites in male broilers reared under regular or cold temperatures at high altitude. Anim. Sci. J. 78:159–163.

Khan, R. U., Z. Nikousefat, M. Javdani, V. Tufarelli, and V. Laudadio. 2011. Zinc-induced moulting: Production and physiology. Worlds Poult. Sci. J. 67:497–506.

Krauze, M., and E. R. Grela. 2010. Effects of an alfalfa concentrate in turkey diets on performance and some blood parameters. Arch. Geflugelkd. 74:226–232.

Laudadio, V., E. Bastoni, M. Introna, and V. Tufarelli. 2013. Production of low-fiber sunflower (*Helianthus annuus* L.) meal by micronization and air classification processes. CyTA—J. Food 11:398–403.

Laudadio, V., E. Ceci, and V. Tufarelli. 2011. Productive traits and meat fatty acid profile of broiler chickens fed diets containing micronized fava beans (*Vicia faba* L. var. minor) as the main protein source. J. Appl. Poult. Res. 20:12–20.

Laudadio, V., and V. Tufarelli. 2010. Treated fava bean (*Vicia faba* var. minor) as substitute for soybean meal in diet of early phase laying hens: Egg-laying performance and egg quality. Poult. Sci. 89:2299–2303.

Laudadio, V., and V. Tufarelli. 2011. Influence of substituting dietary soybean meal for dehulled-micronized lupin (*Lupinus albus* cv. Multitalia) on early phase laying hens production and egg quality. Livest. Sci. 140:184–188.

Mertens, D. R. 2002. Gravimetrical determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. J. AOAC Int. 85:1217–1240.

Mori, A. V., J. R. C. X. Mendonca, C. R. M. Almeida, and M. C. G. Pita. 2003. Supplementing hen diets with vitamins A and E affects egg yolk retinol and α-tocopherol levels. J. Appl. Poult. Res. 12:106–114.

Mourão, J. L., P. I. P. Ponte, J. A. M. Prates, M. S. J. Centeno, L. M. A. Ferreira, M. A. C. Soares, and C. M. G. A. Fontes. 2006. Use of β-glucanases and β-1, 4-xylanases to supplement diets containing alfalfa and rye for laying hens: Effects on bird performance and egg quality. J. Appl. Poult. Res. 15:256–265.

NRC. 1994. Nutrient Requirements for Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

Owusu-Ansah, Y. J., and S. M. McCurdy. 1991. Pea proteins: A review of chemistry, technology of production, and utilization. Food Rev. Int. 7:103–134.

Safaa, H. M., E. Jiménez-Moreno, D. G. Valencia, M. Frikha, M. P. Serrano, and G. G. Mateos. 2009. Effect of main cereal of the diet and particle size of the cereal on productive performance and egg quality of brown egg laying hens in early phase of production. Poult. Sci. 88:608–614.

SAS Institute Inc. 2000. SAS/STAT User's Guide. Release 8.1. SAS Inst. Inc., Cary, NC.

Schweigert, F. J., K. Kreiger, U. Schnurrusch, D. Schams, and J. Gropp. 2002. Effect of dietary β-carotene on the early embryonic development and uterine fluid composition in gilts. J. Anim. Physiol. Anim. Nutr. (Berl.) 86:265–272.

Srinivasan, R., K. B. Hicks, J. Wilson, and R. K. Challa. 2012. Effect of barley roller milling on fractionation of flour using sieving and air classification. Appl. Eng. Agric. 28:225–230.

Srinivasan, R., and V. Singh. 2008. Pericarp fiber separation from corn flour using sieving and air classification. Cereal Chem. 85:27–30.

Surai, A. P., P. F. Surai, W. Steinberg, W. G. Wakeman, B. K. Speake, and N. H. C. Sparks. 2003. Effect of canthaxanthin content of the maternal diet on the antioxidant system of the developing chick. Br. Poult. Sci. 44:612–619.

Surai, P. F. 2002. Natural Antioxidants in Avian Nutrition and Reproduction. Nottingham Univ. Press, Nottingham, UK.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597.

Wu, Y. V., and N. N. Nichols. 2005. Fine grinding and air classification of field pea. Cereal Chem. 82:341–344.