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Research Article

Laying Hen Production and Egg Quality: Short Term Feeding with Sunflower Seed Meal and Probiotics

Yasir Ditta, Sadia Naseem, Ketwee Saksrithai and Annie J King*

Department of Animal Science, University of CA, Davis, CA, USA

***Corresponding Author:** Annie J King, Department of Animal Science, University of CA, Davis, CA, USA.

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Abstract

Horticultural byproducts may provide nutrients for poultry if probiotics can increase digestion of complex carbohydrates. White Leghorns (64 layers at 65- to 74-wk-old) were fed (1) a corn/soy Control, (2) Control + 20% sunflower seed meal (SFM), (3) Control + Probiotics (*Lactobacillus plantarum*, *rhamnosus*, and *paracasei* - each at > 23.3 Mil CFU/g for approximately 70,000,000 Mil CFU/g in chlorinated drinking water), and (4) Control + 20% SFM + Probiotics for four weeks. Significance (P < 0.05, < 0.10) for 4 diets x 4 replications x 4 layers per replication was determined for measurements. Diet did not affect production; fluctuations caused trends for FCR (Probiotics*Week) and feed intake (SFM*Probiotics*Week). SFM increased egg weight, decreased specific gravity, and caused a downward trend for shell thickness. SFM*Week caused temporal changes in specific gravity. Probiotics increased egg and shell weight while decreasing shell thickness; Week produced fluctuations for specific gravity. SFM, SFM*Week, Week, and SFM*Probiotics*Week increased yolk color. Temporal changes occurred by Week and SFM*Week for internal measurements and the Haugh unit, respectively. Future research should assess use of fiber types, digestibility of SFM/Probiotic diets, and colonization of the gut after probiotics in water or feed are fed to various types and ages of layers.

Keywords: *Laying Hens; Probiotics; Sunflower Seed Meal; Growth Performance; Egg Quality*

Introduction

Due to the price variation in soybean meal and corn, poultry nutritionists seek partial or complete replacement for these ingredients with nutritious, lower priced horticultural byproducts in least cost formulations to increase profit margins [1]. Sunflower is a high oil-yielding seed crop which can be cultivated two to three times in tropical areas and is highly adaptive to various climates [2]. Sunflower seed meal (SFM), a rendered byproduct from the sunflower oil industry, is an inexpensive alternate source of vegetable protein and, possibly, energy for animals [2,3]. The crude protein content of SFM, ranging from 29 to 45%, is inversely related to its crude fiber content (14 to 32%) and depends on de-hulling and oil extraction processes [4].

There are limits for use of SFM in feed for poultry and other monogastric animals because its high fiber content is not fully metabolized [5,6]. As well, a lysine deficiency, a low digestion coefficient for lysine, and a negative correlation between fiber content and total metabolizable energy of SFM were reported [4,7]. Enzymes could be added to diets to enhance digestibility of SFM; another possibility is inclusion of probiotics, live microbial non-digestible supplements that can colonize the intestine and ceca of poultry [8].

The major genera of bacteria involved in carbohydrate fermentation are *Bacteroides, Bacillus, Bifidobacterium*, and *Lactobacillus* [9,10]. Upon consumption, lactic acid bacteria are delivered into the gastrointestinal tract where they modify the intestinal milieu; and, if colonized, they increase the levels of amylase needed for catalysis of starch to sugars [11]. They can improve feed efficiency, productivity of laying hens, and egg quality measurements [12]. Due to recent advancements (solvent extraction and de-hulling techniques) in SFM production and use of *Lactobacillus* probiotics for livestock, we investigated their short-term (four week) use, alone and in combination, to determine efficacy of performance and external/internal egg quality for older layers [11-13].

Materials and Methods

Composition of sunflower seed meal and diets

Sunflower seed meal (SFM) was procured from the National Sunflower Association (NSA, Mandan, ND, USA; Table 1). The nutrient composition of the meal was provided by NSA and used in formulation of diets. Ethoxyquin (150 ppm), an antioxidant, was added to SFM during manufacture of the meal. Upon receipt, the meal was immediately stored at -20°C until use.

	Kcal/kg
ME[2] (Kcal/Kg)	1543.20
True ME (Kcal/Kg)	1686.54
Nutrients	**%**
Moisture	10.00
Crude fat	1.80
Crude fiber	25.00
Crude Protein	35.70
Ash	7.15
NFE[3]	20.35
Calcium	0.27
Total Phosphorus	1.10
P (Available)	0.32
Inherent Fat	0.30
Sodium + KCl	0.02
Potassium	1.00
Chloride	0.01
Lysine	1.20
Methionine+ cysteine	1.24
Leucine	1.96
Threonine	1.29

Table 1: *Nutrient composition[1] of sunflower seed meal.*

[1]Supplied by the National Sunflower Association (NSA, Mandan, ND, USA). [2]ME, metabolizable energy; [3]NFE, nitrogen free extract.

Probiotics in drinking water

Sources of (1) water for mixing Probiotics and (2) the viable count of bacteria added were analyzed by Michelson Laboratories, Inc. (Commerce, CA, USA). Available water was double distilled, distilled, and chlorinated. The manufacturer (Living Streams Mission, Athol, Idaho) of Probiotics advised using chlorinated water with $CuSO_4$ (1 ppm) for mixing of bacteria. Heavy metals in chlorinated water were analyzed (UC Davis Analytical Laboratory, Davis, CA). As discussed in Results below, Probiotics - active *Lactobacillus plantarum*, *rhamnosus*, and *paracasei* - were added daily to chlorinated water at > 23.3 Mil CFU/g, totaling approximately 70,000,000 Mil CFU/g.

Hens, diets, and measurements

White Leghorn Crosses (ranging in age from 65- to 74-week-old) were monitored for consistency of weight and egg production. Data were collected for weight (two weeks) and egg production (nine days) prior to beginning the study. Layers of similar weight (1.59 kg, RMS = 0.0804, $P < 0.05$) and egg production of (5.67 eggs per week, RMS = 1.391, $P < 0.05$) were randomly divided into four treatments × four replications × four laying hens/replication and fed either the Control (a corn/soy diet), Control + 20% SFM, Control + Probiotics in drinking water, or Control + 20% SFM + Probiotics in drinking water for four weeks [14, Table 2]. Feed and water were administered *ad libitum*. Layers were singly housed in cages (45.72 cm × 45.72 cm × 53.34 cm). Hens were provided 16 hours of light and 8 hours of darkness throughout the trial, conducted in June and July in Davis, CA. The average temperature in the layer house was 70.19°F ± 4.8.

Feed Ingredients (%)			Nutrients Composition of Feed (%)		
	20% SFM[1]	Control[2]		20% SFM[1]	Control[2]
Corn	52.14	68.48	Moisture	10.79	12.07
SFM	20.00	0.00	ME[4] (Kcal/Kg)	2996.00	2978.76
Limestone	9.65	9.58	True ME (Kcal/Kg)	3141.60	3141.60
Blended fat	8.68	2.89	Crude protein	14.99	13.00
Soybean meal	8.00	17.37	Crude fat	10.27	5.01
Nesfox (MCP)	1.00	1.16	Crude fiber	6.58	2.32
NaCl	0.15	0.15	Ash	12.10	11.48
DL-methionine	0.09	0.16	Arginine	0.96	0.82
L-lysine HCl	0.09	0.00	Lysine	0.64	0.64
Vitamin premix[3]	0.08	0.08	Methionine	0.37	0.37
Choline-HCl	0.08	0.08	Methionine + Cysteine	0.58	0.59
Mineral premix[3]	0.05	0.05	Tryptophan	0.16	0.16
			Leucine	1.08	1.14
			Isoleucine	0.60	0.62
			Threonine	0.53	0.50
			Valine	0.67	0.62
			Calcium	4.00	4.00
			Available Phosphorus	0.31	0.31
			Phytic Phosphorus	0.58	0.49
			Added fat	8.68	2.89
			Sodium	0.15	0.16
			Chloride	0.15	0.15
			Choline (mg/Kg)	0.73	0.57
			Potassium	0.56	0.61
			Linoleic Acid	3.15	2.04
			[5]Na + K – Cl (mEq / Kg)	162.47	180.55

Table 2: *Formulation and nutrients composition of diets containing 20% sunflower seed meal (SFM)[1] and the Control[2].*
[1]Sunflower seed meal (Sunflower Association, Mandan, ND); [2]A corn/soy based diet; [3]Vitamin/minerals premix included vitamin A, 2500 IU; vitamin D_3, 250 IU; vitamin E, 4 IU; vitamin K, 0.4 mg; vitamin B_{12}, 0.004 mg; biotin, 0.08; choline, 875 mg; folacin, 0.21 mg; niacin, 8.3 mg; pantothenic acid, 1.7 mg; pyridoxine, 2.1 mg; riboflavin, 2.1 mg; thiamin, 0.6 mg; iodine, 0.029 mg; iron, 38 mg; manganese, 17; selenium, 0.05; and zinc, 29; [4]ME: Metabolizable energy; [5]Na + K – Cl, dietary anion-cation balance.

Production and egg quality measurements

Weekly feed intake and body weight were measured and the FCR (feed intake/kg dozen eggs) was determined for each diet. Eggs were collected daily and stored at 4°C. Weekly egg quality was measured within 6 to 24 hours after collection. External measurements included egg weight, specific gravity (salt bath method) [15], shape index (egg width/egg length × 100) [16], egg shell weight, and egg shell thickness [17].

Internal measurements included egg yolk color (Roche Yolk Color Fan) [18], egg yolk index (egg yolk height/egg yolk diameter × 100) [19], albumen index (albumen height/average egg albumen width × 100) [20], yolk to albumen ratio (egg yolk weight/weight of albumen) [20], proportional albumen ratio (egg albumen weight/egg weight × 100), and Haugh unit, calculated as:

HU = 100 × log (albumen height - 1.7 + egg weight ^ 0.37 +7.57, [21].

Statistical analysis

A power analysis was conducted to determine the adequate number of layers for this study. With a confidence interval at 50% (for which one-half of the layers were fed Probiotics) and a 95% confidence level, a population of 64 layers was determined to be adequate [22]. Data as a 2 x 2 factorial (Probiotics*SFM, n= 6) in an unbalanced mixed model design with Week as a repeated measure were analyzed using the Proc Mixed Procedure and the PDMIX800SAS Macro [23]. Probiotics (treatment name) was the main effect and SFM (treatment name) became the subplot effect. Pairwise comparisons of treatment means were conducted [23]. The Tukey-Kramer Adjustment was used to assess differences among means at $P < 0.05$. Significance at $P < 0.10$ was noted.

Results

Probiotics in drinking water

Water (1L)	Lactic acid bacteria/L of water[1]	Probiotics 156 uL/L, added and detected[2 +]
Double distilled water A	< 1	-
Double distilled water B	270	+
Distilled water	160	+
Chlorinated water	160	+
Chlorinated water + $CuSO_4$ (1ppm)[3]	13	+

Table 3: *Probiotics in sources of water.*
[1]Bacterial count for all sources of water (as is). [2+] denotes presence of Probiotics as added (156 uL/L) in water = double distilled (B), distilled, chlorinated, and chlorinated + $CuSO_4$. [3]Recommended by Living Streams Living Mission, Athol, Idaho), respectively; no probiotics were added to double distilled A.

As noted in Table 3, lactic acid bacteria was 270 count in double distilled water (not readily available at the grow-out facility) and 160 count in both distilled water from the laboratory and chlorinated drinking water in lines at the grow-out facility (Method COM ED.4 19.521, Michelson Laboratories, Inc., Commerce, CA, USA). Probiotics from the manufacturer were analyzed and found to contain the quantities noted (~56.7g at 4 billion CFU). For ease of mixing and to avoid changes in the mineral content of diets, Probiotics were mixed in chlorinated drinking water, each at > 23.3 Mil CFU/g, totaling approximately 70,000,000 Mil CFU/g. Analysis revealed no concentration of heavy metals to affect the viability of Probiotics in chlorinated drinking water (Table 4).

	Ca	Mg	Na	K	Zn	Cu	Mn	Fe	Cd	Cr	Pb	Ni	Cl	SO_4-
	--------mEq/L[1]-------			-- mg/L[1]--										
	≤0.88	≤1.64	≤3.16	≤2.51	<0.005	<0.01	<0.005	<0.01	<0.005	≤0.011	≤0.015	<0.005	≤0.45	≤10.1
DL[2]	0.01	0.01	0.01	0.05	0.005	0.01	0.005	0.01	0.005	0.005	0.10	0.005	0.1	0.1

Table 4: *Soluble minerals analysis[1] of chlorinated drinking water.*

[1]Analytical Laboratory, UC (Davis, CA, USA). [2]Detection limit.

Production measurements - Table 5

Major Effects[1]	Feed Intake (g/hen/d)	Egg Production (%)	FCR (kg/dozen egg)
SFM[3]			
Control[2]	83.90	65.32	1.60
20% SFM	81.56	61.69	1.63
SE	1.71	1.73	0.05
P value	0.3255	0.1323	0.5909
Probiotics[4]			
Control	81.52	63.19	1.59
156 µL/L	83.94	63.82	1.64
SE	1.79	1.87	0.05
P value	0.3588	0.8207	0.6011
P values			
SFM*Probiotics	0.4448	0.7856	0.4828
Week	0.0011	0.0070	0.0002
SFM*Week	0.9302	0.7391	0.7562
Probiotics*Week	0.1475	0.2034	0.0979
SFM*Probiotics*Week	0.0615	0.5823	0.1063

Table 5: *Major effects[1] of control[2], SFM[3], and Probiotics[4] on production parameters of laying hens during four weeks.*

[1]Means with the same superscript are not significantly different a P < 0.05.

[2]Control (a corn/soy based diet).

[3]Sunflower seed meal.

[4]Probiotics added to provide L. paracasei, L. plantarum, and L. rhamnosus, each at > 23.3 Mil CFU/g, totaling approximately 70,000,000 Mil CFU/g for all added species in chlorinated drinking water.

No mortality occurred during the four week study. SFM, Probiotics, and SFM*Probiotics had no effect on feed intake, egg production, or FCR while Week was significant for all production measurements (Table 5). There were trends due to SFM*Probiotics*Week for feed intake ($p = 0.0615$) and Probiotics*Week for FCR ($p = 0.0979$) caused by fluctuations in measurements over time (Table 5).

External egg quality - Table 6

Major Effects[1]	Egg Weight (g)	Specific Gravity	Shape Index	Egg Shell Weight (g)	Egg Shell Thickness (mm)
SFM[2]					
Control[3]	53.40[b]	1.0842[a]	0.84	5.04	0.35
20%	54.35[a]	1.0826[b]	0.84	5.06	0.34
SE	0.27	4.40×10^{-4}	3.37×10^{-3}	0.02	1.70×10^{-3}
P value	0.0242	0.0082	0.3946	0.6331	0.0581
Probiotics[4]					
Control	53.33[b]	1.0843[a]	0.84	5.02[b]	0.35[a]
156 µL/L	54.42[a]	1.0826[b]	0.84	5.08[a]	0.34[b]
SE	0.28	4.42×10^{-4}	3.37×10^{-3}	0.02	1.72 x 10-^{3}
P value	0.0411	0.0789	0.7799	0.0441	0.0109
P value					
SFM*Probiotics	0.7356	0.0683	0.1819	0.1002	0.9335
Week	0.0010	<0.0001	0.0540	0.0011	0.0280
SFM*Week	0.7595	0.0301	0.9812	0.6710	0.4270
Probiotics*Week	0.6701	0.8936	0.5354	0.9459	0.1550
SFM*Probiotics*Week	0.8510	0.3411	0.5769	0.6181	0.4075

Table 6: *Major effects[1] of control[2], SFM[3] and Probiotics[4] on external quality of eggs from laying hens during four weeks.*
[1]Means with the same superscripts are not significantly different at P < 0.05.
[2]Control (a corn/soy based diet) [3]+ 20% sunflower seed meal (SFM).
[4]Probiotics added to provide each at > 23.3 Mil CFU/g, totaling approximately 70,000,000 Mil CFU/g of each species (L. paracasei, L. plantarum, and L. rhamnosus) in chlorinated drinking water.

SFM significantly increased egg weight (p = 0.0242) while decreasing specific gravity (p = 0.0082). There was a downward trend (p = 0.0581) for eggshell thickness (Table 6). Temporal fluctuations were observed for SFM*Week where specific gravity was significantly affected (p = 0.0301) as shown in Tables 6 and 7 (showing weekly fluctuations). Due to temporal changes, SFM*Probiotics produced a trend for specific gravity at p = 0.0683 and, possibly, shell weight (p=0.1002).

Diet[1]	Week 1	Week 2	Week 3	Week 4
Control[2]	1.084	1.080	1.086	1.087
20% SFM[3]	1.084	1.081	1.083	1.083

Table 7: *Effects of SFM*Week on specific gravity of eggs from 65- to 74-week-old laying hens.*
[1]Means with the same superscript are not significantly different.
Control[2] (a corn/soy based diet) + 20% sunflower seed meal[3](SFM).

For Probiotics, there was a significant increase in egg weight (p = 0.0411) and egg shell weight (p = 0.0441) and a significant decrease in egg shell thickness (p = 0.0109). Specific gravity trended downward at p = 0.0789. Week was significant for egg weight (p = 0.0010), egg shell weight (p = 0.0011), and egg shell thickness (p = 0.0280); it was highly significant for specific gravity (p = < 0.0001) and there was a temporal effect for shape index (p = 0.054). Probiotics*Week and SFM*Probiotics*Week had no effect on external egg quality parameters.

Internal egg quality - Table 8

Major Effects[1]	Egg Yolk Color	Yolk Index	Albumen Index	Yolk:Albumen	Haugh Unit	Proportional Albumen
SFM[3]						
Control[2]	7.55[a]	41.84	6.77	55.46	72.99	57.27
20%	7.34[b]	41.16	6.69	55.63	73.62	57.52
SE	0.05	0.33	0.15	0.56	0.63	0.23
P value	0.0074	0.1521	0.7020	0.8145	0.4943	0.3837
Probiotics[4]						
Control	7.51	41.32	6.73	55.16	73.29	57.53
156µL/L	7.39	41.69	6.74	55.93	73.32	57.26
SE	0.05	0.34	0.15	0.63	0.62	0.25
P value	0.1528	0.4515	0.9503	0.4285	0.9752	0.4723
P value						
SFM*Probiotics	0.7202	0.3551	0.3994	0.7373	0.1219	0.7304
Week	0.0033	<0.0001	0.0046	0.0347	<0.0001	<0.0001
SFM*Week	<0.0001	0.5676	0.4718	0.0254	0.4646	0.0554
Probiotics*Week	0.7752	0.3029	0.3314	0.7948	0.0843	0.7943
SFM*Probiotics*Week	0.0104	0.5959	0.3240	0.3894	0.1426	0.6207

Table 8: *Major effects[1] of control[2], SFM[3] and probiotics[4] on internal quality of eggs from layers fed for four weeks.*
[1]Means with the same superscript are not significantly different.
Control[2] (a corn/soy based diet) + 20% sunflower seed meal (SFM)[3].
[4]Probiotics (L. paracasei, L. plantarum, and L. rhamnosus) added to provide each at > 23.3 Mil CFU/g, totaling approximately 70,000,000 Mil CFU/g for all species in chlorinated drinking water.

Table 8 shows internal quality measurements. SFM significantly decreased (p = 0.0074) yolk color by 2.78%. SFM*Week was highly significant (p = < 0.0001) for yolk color where week 2 (6.97) for 20% SFM was significantly lower when compared to week 1 for the Control (7.64) (Data not shown). SFM*Week was significant (p = 0.0254) for yolk:albumen and caused a trend (p = 0.0554) for proportional albumen due to temporal changes. SFM*Probiotics*Week significantly affected (p = 0.0104) yolk color. Probiotics and SFM*Probiotics had no effects on internal quality. Week was highly significant (p = <0.0001) for yolk index, Haugh unit, and proportional albumen. Also, Week was significant (p = 0.0033) for yolk color, albumen index (p = 0.0046), and yolk:albumen (p = 0.0347).

Discussion

Production: SFM

Our results are in agreement with those of several investigators who found no effect of SFM on production parameters. Uwayjan., *et al*. (1983) and Yalcın., *et al*. (2008) reported no effect of 15% SFM in the diet [24,25]. Shi., *et al*. (2012) found insignificant effects of diets supplemented with a greater quantity of SFM (24.84%, 33% CP) [26]. Laudadio., *et al*. (2014) also reported insignificant effects of SFM (IR, 16%; CP, 42.3%; and CF, 10.3%) on laying hens for weight gain, feed consumption, egg production, and FCR [1]. Our findings and those of other investigators suggest that while SFM did not improve production parameters, there were no detrimental effects. Thus, SFM could be substituted for soybean meal in the diets of older layers.

Production: Probiotics

In our work, there were no effects of Probiotics alone on production parameters while results from other investigators reported improvements. Mohan., *et al*. (1995) reported a quadratic increase in egg production for White Leghorn layers (28 to 38 weeks old) consuming diets supplemented with a mixture of probiotics at 0, 100, and 150 mg probiotics/kg feed [27]. For 100 mg, egg production increased by 5%. Kurtoglu., *et al*. (2004) fed BioPlus, a commercial multi-stain probiotic (250, 500, and 750 mg/kg feed) to 27-week-old Brown Nick layers and showed an increase in egg production [28]. Yőrűk., *et al*. (2004) reported that after 90 days, egg production significantly improved with addition of 0.1% probiotics (*Lactobacillus*, *Bifidobacterium, Streptococcus* and *Enterococcus* species) in the diet [29].

Investigators reported that the average time for colonization of the gut by various *B. Subtilis species* was three to six weeks in older hens [30]. Our results for *Lactobacillus* species seemed to indicate that substantial colonization needed a period longer than 4 weeks to affect production measurements. Alternatively, the quantity of bacterial species used may not have produced significant enhancement for production measurements at or after six weeks. If partial colonization did begin to occur at four weeks in our work, the on-going competition of the *Lactobacillus* species with established organisms may have caused fluctuations in weekly results as discussed below for external and internal egg quality measurements.

As noted above, a lysine deficiency, a low digestion coefficient for lysine, and a negative correlation between fiber content and total metabolizable energy of SFM were reported [4,7]. Probiotics (including the *Lactobacillus* species) as live microbial non-digestible supplements can colonize in the intestine of animals as well as in the ceca of poultry [8]. If colonized, they increase amylase, causing catalysis of starch to sugars [11]. Also, due to enhanced digestibility and absorption of nutrients (including amino acids), they can improve feed efficiency, productivity of laying hens, and egg quality measurements [12]. Due to our hypothesis that Probiotics would improve digestion of SFM fibrous material and amino acids, there was no attempt to adjust digestible lysine and other amino acids across diets. No effect for SFM*Probiotics indicated that *Lactobacillus* species did not greatly enhance production parameters by increasing digestion of lignocellulosic compounds or digestibility of amino acids in SFM. Future work will include results for apparent digestibility of all diets to more accurately measure improvements in amino acid availability caused by *Lactobacillus* species alone and when coupled with SFM.

It was assumed that fluctuations by Week for feed intake and FCR of older layers likely contributed to trends noted for SFM*Probiotic*Week. To further investigate the effects of SFM and Probiotics on layer production parameters, investigations for Probiotics used herein and greater quantities in drinking water and feed of young and older hens should be conducted.

External egg quality: SFM

SFM increased egg weight. Karunajeewa., *et al*. (1989) also reported that SFM increased egg weight of White Leghorn × Australorp hens fed for 24 to 64 weeks [31]. However, Shi., *et al*. (2012) reported that feeding diets supplemented with SFM up to 24.84% for 6 weeks did not influence egg weight [26]. Yalcın., *et al*. (2008) revealed that egg weight of hens fed a SFM or a soybean meal diet with 0 and 2 g/kg commercial yeast culture product (*Saccharomyces cerevisiae*) for 16 weeks did not change [25]. Laudadio., *et al*. (2014) noted that egg weight was statistically similar among eggs of hens fed soybean meal or a low-fiber SFM diet (CP, 42.3%; CF, 10.3% and IR, 16%) for 10 week [1]. These contradictory findings suggest that effect of fiber types and quantities on external egg quality should be thoroughly investigated for various breeds of layers during the entire laying period.

In our study, the decrease in specific gravity caused by SFM and SFM*Week was likely associated with the trend toward a decrease in egg shell thickness and greater egg mass due to age and less calcium deposition [32,33]. While eggshell thickness for SFM trended downward in the present study, it did not fall below 0.33 mm, indicating that eggs had shells of adequate thickness that could withstand the rigors of marketing [34].

SFM had no effect on shape index, generally oval in the range of 72 to 76 [35]. All eggs in our study had an index of 84, indicating roundness and the possibility of breaking if not a proper fit for the standard egg carton [35]. Park found no correlation ($P < 0.05$) between shape index and breaking strength (not associated with the carton size) while Sarica., *et al*. noted a positive correlation [35,36].

External egg quality: Probiotics

Our finding for an increase in egg weight produced by Probiotics did not agree with those of Kurtoglu., *et al*. (2004) who noted no effect [28]. As Probiotics increased egg weight, eggshell weight increased and eggshell thickness decreased. For 100 mg probiotics/kg diet, Mohan., *et al*. (1995) also reported fewer thin-shelled eggs (8.6%) compared to the control (18.6%). Relative to increased egg weight, investigators suggested that probiotics promoted growth of intestinal microflora, thereby producing a healthy gut lining causing improved digestion and subsequent deposition of nutrients in yolk (thus, greater weight) although mechanisms are not understood [37]. A decrease in eggshell thickness was validated by the trend toward a decline in specific gravity [33]. SFM*Probiotics affected specific gravity and eggshell weight; also, these measurements may have been influenced by the age of hens.

Internal egg quality: SFM

Egg yolk color was decreased by SFM, Week, SFM*Week, and SFM*Probiotics*Week. The decrease in color caused by SFM and temporal effects were likely due to dilution of dietary pigments in corn, the major contributor of fat soluble carotenoids, by SFM [38,39]. Fat at 2X more in the SFM diet than in the Control may have caused some dilution of color as well.

The significant effect of SFM*Week on the yolk:albumen and proportional albumen may have indicated greater internal weight of the egg associated with increasing age. The Haugh unit, the standard for overall internal egg quality, remained low throughout the study. A Haugh unit for young layers is > 91; that for older layers in our work was ~73, lower than that (81.39) of 70-week-old ISA brown layers [40]. Researchers note that age is an important factor causing a decline in albumin quality, associated with Haugh units [41]. As well, in the present work, fluctuation in Haugh units over time, as with some production and egg quality measurements, may have been related to intermittent colonization of *Lactobacillus* species during the 4-week period.

Internal egg quality: Probiotics

Improved digestion of nutrients and thereby egg weight has been associated with probiotics as noted above for external quality measurements [37]. However, in our work, the effect for Probiotics seems more likely associated with the thickness of egg shell and not an increase in internal quality measurements. More work with younger birds and/or greater quantities of Probiotics may help clarify these findings.

Conclusion

Neither SFM, Probiotics, nor SFM*Probiotics affected production measurements. SFM increased egg weight and decreased specific gravity. SFM caused a decrease in eggshell thickness, likely compounded by less calcium deposition as hens aged. A decrease in yolk color was likely due to addition of SFM that reduced pigments; increased fat content in SFM diets likely caused a diluting effect as well. SFM*Probiotics caused changes in external egg quality measurements at $P < 0.01$, but had no effect on internal egg quality. Week (age of hens) affected production and external/internal measurements. More work is needed to clearly establish (1) the effect of various fiber types on production and egg quality parameters, (2) the digestibility of SFM/Probiotic diets, and (3) time needed for colonization of various quantities of probiotics (added in water and feed) in the gut of various breeds and ages of laying hens.

Author Contributions

Conceptualization, Annie King, Yasir Ditta, Ketwee Sakrithai, and Sadia Naseem; and Ketwee Saksrithai; Formal analysis, Yasir Ditta, Sadia Naseem and Ketwee Saksrithai; Investigation, Annie King, Yasir Ditta, Sadia Naseem and Ketwee Saksrithai; Methodology, Annie King, Yasir Ditta, Sadia Naseem and Ketwee Saksrithai; Project administration, Annie King; Resources, Annie King; Supervision, Annie King; Writing - original draft, Yasir Ditta; Writing - review and editing, Annie King.

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Institutional Review Board Statement

The Institutional Animal Care and Use Committee (UC Davis, Davis, CA) approved the protocol (#21596) for feeding and care of laying hens.

Data Availability

https://datadryad.org/stash/share/9RAi9ukt46JYST26C6xVFo0em-lbcmN4swKL4z_7ygE.

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Conflicts of Interest

The authors declare no conflicts of interest.

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