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The Effects of Vitamin D Supplementation to Peak-producing Hens Fed Diets Differing in Fat Source and Level on Laying Performance, Metabolic Profile, and Egg Quality

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ABSTRACT : This experiment was designed to examine the effects of supplemental vitamin D on laying performance, metabolic profile and egg quality of hens fed diets containing different fat sources and levels. Lohman strains (n = 480) were assigned to one of 10 diets: basal diet (BD), BD plus 2.5 and 5.0% sunflower oil (SO) or tallow (T) at vitamin D provided 1× and 3× of the current recommendation. The experiment lasted from week 30 to 44 of age. Each diet was tested in 12 replicate cages of 4 hens. Production, metabolism, and egg quality data were subjected to three-way ANOVA. Both fats decreased feed intake (FI) as compared to BD. Increasing SO and T levels linearly decreased and quadratically increased FI, respectively. The dietary factors did not affect egg production (EP) and egg weight. Vitamin D supplementation increased and decreased EP when diets contained SO and T, respectively. Feed conversion efficiency (FCE) for hens fed SO was lower than for hens fed T. However, increasing T level improved FCE, whereas increasing SO level worsened FCR. Vitamin D supplementation increased serum vitamin D and glucose concentrations. Vitamin D supplementation also caused a decrease and an increase in serum vitamin D concentration when diets contained SO and T, respectively. Serum glucose concentration for hens fed SO was lower than hens fed T. Increasing fat level linearly increased serum triglyceride and VLDL concentrations, regardless of the fat type. Increasing SO level linearly decreased serum cholesterol concentration. Vitamin D supplementation did not alter lipid metabolites. The dietary factors did not affect serum total protein, Ca, and P concentrations. As compared with BD, feeding SO decreased dry tibia and ash weights more than feeding T. Vitamin D supplementation tended to increase dry tibia weight and decrease tibia ash weight. Eggshell strength and thickness, yolk and albumen indexes, and Haugh unit were not responsive to the dietary factors. Eggshell strength quadratically increased with increasing T level. Yolk color for hens fed SO was lower than for hens fed T. The dietary factors did not affect most of yolk fatty acids. Increasing SO level quadratically decreased yolk $C_{18:2}$ concentration. Vitamin D supplementation increased and decreased yolk $C_{18:2}$ concentration when diets contained SO and T, respectively. In conclusion, increasing fat level improved laying performance without altering metabolic profile and egg quality. Vitamin D supplementation had minor alteration effects on laying performance, metabolic profile, and egg quality in response to fat feeding.
(**Key Words :** Sunflower Oil, Tallow, Vitamin D, Hen, Production, Metabolism, Egg Characteristics)

INTRODUCTION

Fat is a generic term and commonly included in poultry diets to increase the energy density and reduce dustiness of feed. The energy content of fats varies depending upon their chemical structures. The degree of unsaturation and chain length affect metabolism (Manilla et al., 1999; Sanz et al., 2000), digestibility and absorbability of fats (Moran, 1989) and consequently, their metabolizable energy value (Fuller and Rendon, 1977). Animal-origin fats have lower iodine value and higher absorbability than plant-origin fats (Renner and Hill, 1960). Iodine value is an indicator of the degree of unsaturation; it is 48 and 113 for tallow and sunflower oil, respectively.

Vitamin D is a pro-hormone and is required for the production of 1,25-dihydroxyvitamin D, the active form. Calcium and phosphorus homeostasis regulatory effect of vitamin D is similar to that of parathyroid hormone. It stimulates bone calcium mobilization and inhibits urinary calcium excretion when hens are calcium deficient or need

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more calcium, through molecular mechanisms involving bone cells (Sugiyama and Kusuhara, 2001), vitamin D binding proteins (Soares et al., 1995) such as calbindin (Bar et al., 1999; Yosefi et al., 2003) and ovocleidin-17 (Nys et al., 1999) as well as other hormones such as estrogen and thyroxin (van Leeuwen et al., 2001). In addition to these physiological effects, unlike parathyroid hormone, vitamin D stimulates absorption of dietary calcium through the intestine to maintain normocalcemia. Due to extensively confined housing system and a greater calcium output via eggshell mass relative to body size and intake, efficient utilization of dietary calcium is crucial for bone health, laying performance, and egg quality (Mitchell et al., 1997; Wesbter, 2004).

Fat supplementation has received enormous attention in poultry feeding, mostly with respect to broiler performance due to substantial high-energy requirement as well as quality of meat (Kırkpınar et al., 1999; Manilla et al., 1999; Newman et al., 2002; Azman et al., 2005; Schreiner et al., 2005) and eggs (Grobas et al., 2001) as reflected by "functional food for human nutrition". Fat feeding however is associated with reduced calcium and magnesium retentions (Leeson and Atteh, 1995) and increased digesta transit time (Dänicke et al., 1999). After digestion of fats, released fatty acids form insoluble soaps with intestinal cations (Whitehead et al., 1971), which may compromise bioavailability of dietary calcium and other nutrients. To maintain absorbable calcium, increasing dietary calcium levels failed to alter the adverse effect of fat feeding (Smith et al., 2003). The addition of vitamin D into diets containing fat, with respect to calcium metabolism, has not been elaborately studied in detail. It was hypothesized that fat feeding may attenuate calcium metabolism and consequently, worsen bone health and reduce outer eggshell quality. Thus, fat feeding may increase demand for vitamin D. The objective of this experiment therefore was to examine the effects of vitamin D supplementation to diets of hens containing different levels and sources of fat on laying performance, metabolic profile, and egg quality during the peak production period.

MATERIALS AND METHODS

Animal, diet, and management

The Research Animal Ethic Committee of Atatürk

Table 1. Ingredient and chemical composition of the experimental diets

Ingredient (%)	Experimental diets[1]				
	BD	2.5FO	5.0FO	2.5T	5.0T
Corn	51.00	47.60	44.10	47.60	44.10
Soybean meal (44% CP)	23.00	23.70	24.40	23.70	24.40
Wheat	6.00	6.00	6.00	6.00	6.00
Barley	2.50	2.50	2.50	2.50	2.50
Wheat bran	9.30	9.40	9.50	9.40	9.50
Molasses	1.80	1.80	1.80	1.80	1.80
Sunflower oil	-	2.50	5.00	-	-
Tallow	-	-	-	2.50	5.00
Limestone	4.50	4.50	4.50	4.50	4.50
Salt	0.30	0.30	0.30	0.30	0.30
Dicalcium phosphate[2]	1.10	1.10	1.10	1.10	1.10
Vitamin-mineral premix[3]	0.20	0.20	0.20	0.20	0.20
Antioxidant[4]	0.10	0.20	0.40	0.20	0.40
DL-methionine	0.10	0.10	0.10	0.10	0.10
L-lysine.HCl	0.10	0.10	0.10	0.10	0.10
Chemical composition					
ME (kcal/kg)[5]	2,726.00	2,832.00	2,934.00	2,823.00	2,916.00
Crude protein (%)	16.44	16.58	16.61	16.58	16.61
Crude fiber (%)	3.84	3.82	3.79	3.82	3.79
Ether extract (%)	2.61	4.94	7.35	4.91	7.16
Ca (%)[5]	2.35	2.35	2.35	2.35	2.35
P (%)[5]	0.59	0.59	0.59	0.59	0.59

[1] Diets: BD = basal diet; 2.5SO = diet containing 2.5% sunflower oil; 5.0SO = diet containing 5.0% sunflower oil; 2.5T = diet containing 2.5% tallow; 5.0T = diet containing 5.0% tallow. The other diets that have similar ingredients and were supplemented with vitamin D (Lutavit® D_3 500, 500,000 IU cholecalciferol/g, BASF Aktiengesellschaft, Ludwigshafen/Germany) to exceed vitamin D requirement by 3-fold at expense of wheat bran and they were BDD, 2.5SOD, 5.0SOD, 2.5TD, and 5.0TD, respectively.

[2] Per kg contains: Ca, 24% and P, 17.5%.

[3] Per kg contains: Vitamin A, 1,500,000 IU; cholecalciferol, 150,000 ICU, vitamin E (dl-α-tocopheryl acetate), 3,000 IU; menadione, 50.0 mg; thiamine, 30.0 mg; riboflavin, 60.0 mg; niacin, 200.0 mg; panthotenic acid, 80.0 mg; pyridoxine, 50.0 mg; folic acid, 10.0 mg; vitamin B_{12}, 150.0 mcg; Mn, 800.0 mg; Zn, 600.0 mg; Fe, 300.0 mg; Cu, 50.0 mg; I, 20.0 mg; and Se, 1.5 mg.

[4] Per kg contains: Ethoxyquin, 1.26 g.

[5] Calculated values (Jurgens, 1996).

University approved all procedures under this experimental protocol. Lohman strains (n = 480), 30 wks of age with uniformity of 93% (the number of hens weighing within 0.9-1.1% of the mean body weight), were blocked according to the cage location. Birds were then assigned randomly to one of 10 experimental diets for a period of 14 weeks. The experimental diets were basal diet not containing fat, basal diet plus 2.5 and 5.0% either sunflower oil or tallow, and these diets supplemented with additional vitamin D. The first 5 diets included to vitamin D at recommended level (NRC, 1994). Supplemental vitamin D (Lutavit® D_3 500, 500,000 IU cholecalciferol/g, Lot #: JAF 2 086903/1-26, BASF Aktiengesellschaft, Ludwigshafen/ Germany) was included in the last 5 diets at the expense of wheat bran to provide 3 folds of the NRC recommendation (NRC, 1994). Each diet was offered to 12 replicated cages (50×46×46 cm, width x depth x height) of 4 hens. The experimental diets (Table 1), isonitrogenous but not isocaloric, were formulated to meet or exceed the NRC recommendations (NRC, 1994). Hens were fed *ad libitum* once daily at 08:00 h, water was always available, and the hen house was lit for 17 h during the experimental period, from week 30 to 44 of age.

Sample collection and analytical procedure

Feed samples were analyzed for dry matter, crude protein, crude fiber, and ether extract concentrations (AOAC, 1990). Apparent metabolizable energy, Ca, and P contents of the experimental diets were calculated from tabular values of feedstuffs for chickens reported by Jurgens (1996). Feed intake and egg production were recorded daily; egg weight was measured bi-weekly; and body weight was measured before and after the experimental period. Feed conversion efficiency (FCE) was expressed as kilogram feed intake per kilogram egg production.

At the end of the experimental period, blood samples were collected from the brachial vein of two hens from each cage and put into additive-free vacutainers. Serum was obtained following centrifugation at 3,000 g for 15 min at 20°C. Aliquots were kept at -20°C until laboratory analyses for glucose, total protein, albumin, creatine, triglyceride, cholesterol, very low-density lipoprotein (VLDL), Ca, and P using commercial kits (DDS® Spectrophotometric Kits, Diasis Diagnostic Systems Co., İstanbul 80270, Turkey) as well as vitamin D using RIA (ALPCO Diagnostic™ Immunoassay Kits, Catalog #: 38-KIP-1921, Salem, NH, USA). Intra-assay CV was 10.9% in hormone analysis.

A sample of 24 eggs was randomly collected from each experimental group every month to assess egg quality parameters using the following formulas as described by Ergün et al. (1987): Shape index (%) = (egg width, cm/egg length, cm)×100; shell strength (kg/cm×cm) was determined by using a spiral pressure system; shell thickness (mm) was determined in 3 different parts (upper and lower ends and middle) by using micrometer; albumen index (%) = (albumen height, mm/average of albumen length, mm and albumen width, mm)×100; yolk index (%) = (yolk height, mm/yolk diameter, mm)×100; yolk color was determined by using commercially available yolk color fan according to the CIE standard colorimetric system; Haugh unit = $100\times\log (H+7.57-1.7\ W^{0.37})$, where H = albumen height (mm) and W = egg weight (g).

Six hens from each experimental group were sacrificed at the end of the experiment for bone characteristics. After excising and removing flesh, the left tibias were dried overnight at 100°C. Samples were ashed at 540°C for 8 h.

Two eggs from each cage were also collected at the end of the experimental period for fatty acid profile. After extracting fat (Folch et al., 1957), fat and yolk samples were methylated for gas chromatographic analysis (GC-17A version 3, Shimadzu chromatograph 6 equipped with a flame ionization detector, a capillary column, and a film thickness of 0.25 μm). Oven temperature was from 120 to 220 °C at 4 °C/min, from 148 to 158°C at 2.5°C/min, and from 158 to 225°C at 5°C/min. Injector and detector temperature was 280°C; head pressure was 8.7 psi; sample volume was 0.4 μl for fats and 0.5 μl for yolk samples.

Statistics

In a randomized block design experiment, the location of cages (cages at upper or lower level and/or by window or corridor side) was considered as a blocking factor due to a possibility of differences in airflow and light-intensity. Feed intake and egg production data were reduced to bi-weekly means by hens of cage before statistical analyses. Body weight data measured prior to initiation of the experiment was used as a covariate for statistical analyses of all response variables. Analysis of covariance was conducted using the Mixed Procedure (SAS, 1998) as repeated measures with first-order autoregressive covariance structure with time being subplot (Littell et al., 1996). The linear model to test the effects of the experimental diets on laying performance and egg quality parameters was as follows: $Y_{aijk} = \mu+b_0+ b_1(Cov_a)+B_i+FT_j+FL_k+VD_l+(FT*FL)_{jk}+(FT*VD)_{jl}+(FL*VD)_{kl}+(FT*FL*VD)_{jkl}+T_{mj}+$Error A$+(FT*T)_{jm}+(FL*T)_{km}+(VD*T)_{lm}+(FT*FL*T)_{jkm}+(FT*VD*T)_{jlm}+(FL*VD*)_{klm}+(FT*FL*VD*T)_{jklm}+$Error B, where Y_{aijk} = response variable, μ = population mean, b_0 = intercept, b_1 = slop,. Cov_a = covariate (a = body weight), B_i = block (i = 1 cage at lower level by corridor side to 6 cage at upper level by window side), FT_j = fat type (j = diet sunflower oil or tallow), FL_k = fat level (k = 0 to 5.0%), VD_l = vitamin D level (l = 1 or 3-fold of NRC recommendation), corresponding interactions among dietary factors, T_m = time (m = week relative to initiation of

the experiment), Error A = whole plot error, corresponding interactions of dietary factors with time, and Error B = subplot error.

Because of having sampled only once during the experimental period, time factor was omitted from the linear model for analyses of blood metabolites and yolk fatty acids. A polynomial contrast was constructed to determine the nature of response variables to increasing levels of different fat sources. The effects of experimental diets on response variables were considered to be significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Fatty acid profile of treatments and vitamin D supplementation

In general, animal-origin fat sources are richer in $C_{14:0}$, $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$ and poorer in $C_{18:2}$ and other longer chain fatty acids than plant-origin fat sources. Concentrations of $C_{14:0}$, $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$, and other longer chain fatty acids are reported to be 0, 5.4, 0.2, 3.5, 45.3, 39.8, 0.2, and 5.6 g/100 g total fatty acids in sunflower oil and 3.0, 24.5, 3.7, 19.3, 40.9,3.2, 0.7, and 4.9 g/100 g total fatty acids in tallow, respectively (NRC, 2001). These values are highly variable depending upon various factors such as animal age, diets fed to animal, and seed variety. In the present experiment, concentrations (% of total fatty acids) of $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$, C18:2, C18:3, total unsaturated fatty acids, and saturated fatty acids were 10, 0.3, 4.9, 27.0, 58.2, 1.1, 85.0, and 14.0 for sunflower oil and 19.0, 1.9, 9.0, 39.0, 28.0, 1.6, 69.0, and 29 for tallow, respectively. Laying hens are responsive to dietary fat treatments. Fat feeding may reduce fatty acid uptake of yolk from the liver. Unlike in ruminants (Sasaki et al., 2001), due to a lack of biohydrogenation in hens, unsaturated fatty acids are incorporated into tissue or yolk fat without modification. Saturated fatty acids, however, undergo desaturation, elongation, and further desaturation processes in the liver, for example being $C_{18:0}$ converted to $C_{18:1}$, and then possibly to $C_{18:2}$, and $C_{18:3}$ (NRC, 1994).

NRC (1994) recommends that laying hen diets should provide 33 ICU of vitamin D_3 at 110 gram of feed intake. The basal diet in this experiment met the current vitamin D requirement (NRC, 1994). By adding extra vitamin D at the expense of wheat bran, half of the experimental diets provided as much as 3-fold of the current NRC recommendation. This increase is still within the pharmacological dose without causing hypervitaminosis D

Table 2. The effects of additional vitamin D in the diets of peak producing hens fed different sources and levels of fat on laying performance

Dietary factors[1]			Response variables			
Fat source	Fat level	Vitamin D level	Feed intake (g/d)	Egg production (%)	Egg weight (g)	Feed conversion efficiency (kg feed:kg egg)
No Fat	0%	1×	126.4	88.4	67.93	1.94
		3×	122.1	83.9	66.81	1.81
Sunflower oil	2.5%	1×	122.0	87.6	67.07	1.83
		3×	123.3	90.6	66.39	1.87
	5.0%	1×	120.4	90.0	65.50	1.89
		3×	119.4	90.6	65.24	1.89
Tallow	2.5%	1×	126.1	92.3	65.95	1.96
		3×	126.2	91.4	66.80	1.94
	5.0%	1×	120.5	89.7	67.29	1.86
		3×	123.2	87.9	67.29	1.88
SEM			1.2	1.1	0.91	0.02
ANOVA			---------- p<F ----------			
Fat source			0.01	0.44	0.24	0.02
Fat level			0.001	0.26	0.74	0.17
Linear effect of sunflower oil			0.008	0.001	0.02	0.58
Quadratic effect of sunflower oil			0.67	0.41	0.65	0.25
Linear effect of tallow			0.11	0.02	0.92	0.96
Quadratic effect of tallow			0.02	0.0001	0.25	0.0001
Vitamin D level			0.48	0.12	0.56	0.06
Fat source×fat level			0.46	0.007	0.08	0.001
Fat source×vitamin D level			0.56	0.05	0.49	0.59
Fat level×vitamin D level			0.94	0.29	0.86	0.94
Fat source×fat level×vitamin D level			0.24	0.64	0.60	0.21

[1] The experimental diets were basal diet not containing fat and basal diet plus 2.5 and 5.0% either sunflower oil or tallow with meeting (1×) and exceeding NRC recommendation (NRC, 1994) by 3-fold (3×).

(Terry et al., 1999; Fritts and Waldoup, 2003). Moreover, the dietary calcium concentration was maintained to be constant across the experimental diets (Table 1).

Despite known effects of fat on physiology of gastrointestinal tract and metabolism of other nutrients, it is not known whether the dietary level of vitamin D should be increased when fats are fed to laying hens. Several studies dealing with fat feeding have primarily focused on the direct effect of fats on animal performance and meat quality. This experiment addresses whether fat feeding compromises calcium availability and increases demand for dietary vitamin D, assessing serum vitamin D and calcium concentrations and outer eggshell parameters.

Laying performance

Initial (1.64 kg) and final (1.73 kg) body weights of hens were not different across the dietary treatments (data not shown). Regardless of dietary factors, all hens gained an average of 5.57% weight. Moreover, there was no effect of interactions among dietary factors on relative body weight change. However, increasing tallow level but not sunflower oil level increased relative body weight gain quadratically ($p<0.02$). Because of greater energy need, most fat feeding studies have involved broilers. In agreement with body weight gain response in this study, Smith et al. (2003) showed that tallow resulted in greater daily gain than corn oil fed to broilers.

Table 2 summarizes the effect of feeding different sources and levels of fat with or without additional vitamin D on laying performance. All laying performance variables fluctuated during experiment (time effect, $p<0.0001$). Feeding different fat sources ($p<0.01$) and levels ($p<0.001$) affected feed intake. Compared to the basal diet (124.2 g/d), feed intake of hens fed sunflower oil and tallow decreased by 2.37 and 0.16%, respectively. Moreover, feed intake decreased linearly ($p<0.008$) and increased quadratically ($p<0.02$) with increasing feeding level of sunflower oil and tallow, respectively. There were no interaction effects of dietary factors on feed intake. Unlike the present study, other studies dealing with fats (tallow vs. sunflower oil) fed to broilers (Newman et al., 2002) and layers (Baucells et al., 2000) showed no changes in feed intake. The reduction in feed intake could be linked to elevated energy density (Sell et al., 1987; Grobas et al., 2001). The greater depression in feed intake of hens fed sunflower oil, compared with hens fed tallow could be attributed to differences in proportion of saturated and unsaturated fatty acids (Renner and Hill, 1960; Grobas et al., 2001), which influence digestibility, absorbability, and passage rate of nutrients.

There was no main effect of dietary factors on hen-day egg production. With increasing the level of sunflower oil and tallow, hen-day egg production increased linearly and quadratically, respectively ($p<0.0001$ for both). Increasing sunflower oil from 2.5 to 5.0% increased hen-day egg production from 89.1 to 90.3%, whereas increasing tallow from 2.5 to 5.0% decreased hen-day egg production from 91.8 to 88.8%, respectively ($p<0.007$). Vitamin D supplementation increased hen-day egg production from 88.8 to 90.6% when diet included sunflower oil, whereas it decreased from 91.0 to 89.7% when diet contained tallow ($p<0.05$). Paik et al. (2005) who supplemented late-laying ISA Brown strains with 4,000 IU of vitamin D also reported increases in egg production and egg weight and decreases in defective egg percentage. A lack of egg production responses to fat feeding (Grobas et al., 2001; Baucells et al., 2002; Steinhilber, 2005) and vitamin D (Mattila et al., 2004) is consistent with literature.

Egg weight was independent from the dietary factors. However, increasing sunflower oil level was associated with a linear decrease in egg weight ($p<0.02$). Moreover, egg weight tented to increase and decrease with increasing tallow and sunflower oil levels, respectively ($p<0.08$). As reported by Sell et al. (1987) and Grobas et al. (2001), an increase in egg weight in response to supplemental fat in the literature could be related to the increased energy density of diet. Some researchers (Balnave, 1970; Guenter et al., 1971) postulated that this response is linked to the partial alleviation of linoleic acid deficiency. In this study, however, egg weight did not increase in response to increasing sunflower oil.

Feed conversion efficiency (kg feed/kg egg) for hens fed sunflower oil was lower than hens fed tallow (1.87 vs. 1.91; $p<0.02$). Increasing tallow level from 2.5 to 5.0% improved FCE by 4.1%, whereas increasing sunflower oil from 2.5 to 5.0% worsened FCE by 2.2% ($p<0.001$). Consequently, FCE improved quadratically ($p<0.0001$) as tallow level increased. Vitamin D supplementation tended to improve FCE. Smith et al. (2003) fed broilers diets containing 4.7% corn oil and tallow at 0.93 or 1.50% calcium and reported a lack of main effect of fat type and calcium level and fat type by calcium level interaction effects on FCE. Improvement in FCE in response to fat feeding is due to decrease in feed intake and no changes in egg production and egg weight.

Metabolic profile and bone characteristics

The effects of the experimental diets on blood metabolites are presented in Table 3. Expectedly, vitamin D supplementation increased serum vitamin D concentration by 1.26 folds ($p<0.01$). Supplementation of vitamin D was associated with a 17.6% decrease and a 48.1% increase in serum vitamin D concentration when the diet contained sunflower oil and tallow, respectively ($p<0.03$). Moreover, the increase in serum vitamin D concentration in response vitamin D supplementation tended to decrease quadratically with increasing fat level ($p<0.07$). This could be attributed

Table 3. The effects of additional vitamin D in the diets of peak producing hens fed different sources and levels of fat on metabolic profile

Dietary factors[1]			Response variables[2]									
Fat source	Fat level	Vitamin D level	VitD	Glc	TP	Alb	Cre	TG	Chol	VLVL	Ca	P
No fat	0%	1×	32.67	266.8	6.13	2.02	0.35	790.8	173.8	158.2	16.0	5.52
		3×	71.00	276.5	6.75	2.00	0.40	783.3	225.3	156.5	16.3	6.83
Sunflower oil	2.5%	1×	66.48	277.5	7.02	2.12	0.47	796.5	198.7	159.3	16.4	7.15
		3×	38.84	301.5	6.60	2.02	0.50	806.2	169.2	161.3	16.2	6.07
	5.0%	1×	40.82	291.3	7.08	2.27	0.52	814.0	159.2	162.8	16.1	6.93
		3×	49.59	294.3	6.85	2.25	0.48	818.7	144.5	163.7	16.0	6.88
Tallow	2.5%	1×	40.58	304.7	7.13	2.23	0.52	812.0	179.7	162.5	16.4	7.70
		3×	54.50	307.3	7.52	2.25	0.45	808.8	199.7	161.7	16.3	8.15
	5.0%	1×	36.47	290.8	6.50	2.07	0.43	810.7	163.3	162.2	16.2	6.13
		3×	59.58	310.0	7.43	2.23	0.43	821.2	227.3	164.3	16.1	9.02
SEM			8.70	8.4	0.38	0.08	0.04	4.5	29.6	0.9	0.1	0.96
ANOVA			---------- p<F ----------									
Fat source			0.85	0.05	0.33	0.58	0.19	0.18	0.24	0.17	0.47	0.15
Fat level			0.57	0.85	0.71	0.40	0.51	0.002	0.53	0.002	0.06	0.97
Linear effect of sunflower oil			0.55	0.02	0.10	0.003	0.0001	0.0001	0.05	0.0001	0.66	0.27
Quadratic effect of sunflower oil			0.67	0.32	0.70	0.32	0.04	0.92	0.693	0.96	0.23	0.91
Linear effect of tallow			0.66	0.001	0.23	0.11	0.16	0.0001	0.90	0.0001	0.98	0.22
Quadratic effect of tallow			0.75	0.006	0.10	0.04	0.03	0.03	0.79	0.03	0.09	0.29
Vitamin D level			0.01	0.04	0.22	0.90	0.90	0.58	0.26	0.67	0.98	0.22
Fat source×fat level			0.52	0.46	0.33	0.02	0.19	0.14	0.37	0.17	0.94	0.63
Fat source×vitamin D level			0.03	0.83	0.07	0.21	0.51	0.58	0.13	0.55	0.75	0.09
Fat level×vitamin D level			0.07	0.85	0.49	0.33	0.99	0.50	0.49	0.47	0.94	0.20
Fat source×fat level×vitamin D level			0.27	0.12	0.73	0.78	0.19	0.15	0.73	0.10	0.63	0.61

[1] The experimental diets were basal diet not containing fat and basal diet plus 2.5 and 5.0% either sunflower oil or tallow with meeting (1×) and exceeding NRC recommendation (NRC, 1994) by 3-fold (3×).

[2] Response variable: VitD = vitamin D; Glc = glucose; TP = total protein; Alb = albumin; Cre = creatine; TG = triglyceride; Chol = cholesterol; VLDL = very low-density lipoprotein. Unit for all parameters is mg/dl, except for vitamin D (pg/ml).

to the fact that fats also affect absorbability of fat-soluble nutrients (Mazalli et al., 2004).

Serum glucose concentrations for hens fed sunflower oil was lower than hens fed tallow (291 vs. 303 mg/dl; $p<0.05$). Increasing vitamin D level by 3-fold resulted in a 4.1% increase in serum glucose concentration ($p<0.04$). Moreover, increasing both sunflower ($p<0.02$) and tallow ($p<0.001$) levels linearly increased serum glucose concentration. The effect of fat on blood glucose seems to indirectly relate to hormonal alteration. Fat feeding decreases serum insulin concentration but increases insulin-stimulated glucose uptake (Luo et al., 1996).

None of the dietary factors affected serum total protein concentration. Serum albumin concentration linearly increased with increasing levels of sunflower oil ($p<0.003$) and tallow ($p<0.04$). Despite a lack of main effects, there was a significant fat source by fat level interaction effect on serum albumin concentration ($p<0.02$). Increasing the level of sunflower oil and tallow from 2.5 to 5.0% was associated with a decrease and an increase in serum albumin concentrations, respectively. There were no main effects and interaction effects of dietary factors on serum creatine concentration. There were linear and quadratic increases in serum creatine concentrations when levels of sunflower oil ($p<0.0001$) and tallow ($p<0.03$) were increased from 2.5 to 5.0%, respectively. The effect of fat feeding on protein metabolites is not clear. However, several studies negated dietary energy and protein on a proportional basis (NRC, 1994). In this study, ratio of energy to protein decreased as fat level increased.

Fat level, but not fat type and vitamin D supplementation affected serum triglyceride and VLDL concentrations ($p<0.002$ for both). Regardless of fat type, increasing fat level linearly increased serum triglyceride and VLDL concentrations ($p<0.0001$ for both). Interestingly, serum cholesterol concentration was independent from the fat type and was variably affected by fat level. Increasing sunflower oil linearly decreased serum cholesterol concentration ($p<0.05$). It is well established that hens fed unsaturated fatty acids have lower serum triglyceride and cholesterol concentrations than hens fed saturated fatty acids (Newman et al., 2002; Özdoğan and Akşit, 2003).

There were no effects of the experimental diets on serum Ca and P concentrations. Increasing tallow level tended to decrease serum Ca concentration quadratically ($p<0.09$). Vitamin D supplementation tended to decrease and increase serum P concentrations with increasing levels of sunflower oil and tallow, respectively ($p<0.09$). A lack of

Table 4. The effects of additional vitamin D in the diets of peak producing hens fed different sources and levels of fat on bone characteristics

Dietary factors[1]			Tibia characteristics		
Fat source	Fat level	Vitamin D level	Dry weight (g)	Ash weight (g)	Ash (%)
No Fat	0%	1×	5.58	2.51	44.96
		3×	6.31	2.71	42.95
Sunflower oil	2.5%	1×	5.33	2.42	45.34
		3×	5.21	2.28	43.90
	5.0%	1×	5.54	2.48	44.69
		3×	5.97	2.58	43.28
Tallow	2.5%	1×	5.87	2.58	44.04
		3×	6.28	2.73	43.33
	5.0%	1×	5.48	2.46	44.96
		3×	5.96	2.55	42.73
SEM			0.24	0.12	1.11
ANOVA			---------- p<F ----------		
Fat source			0.05	0.09	0.90
Fat level			0.81	0.65	0.64
Linear effect of sunflower oil			0.79	0.65	0.81
Quadratic effect of sunflower oil			0.27	0.31	0.75
Linear effect of tallow			0.91	0.46	0.93
Quadratic effect of tallow			0.23	0.21	0.89
Vitamin D level			0.07	0.55	0.04
Fat source×fat level			0.25	0.46	0.48
Fat source×vitamin D level			0.29	0.27	0.74
Fat level×vitamin D level			0.89	0.87	0.55
Fat source×fat level×vitamin D level			0.73	0.81	0.88

[1] The experimental diets were basal diet not containing fat and basal diet added 2.5 and 5.0% either sunflower oil or tallow with meeting (1×) and exceeding NRC recommendation (NRC, 1994) by 3-fold (3×).

fat type, but calcium level effect on serum calcium concentrations is reported in broilers fed 4.7% corn oil and tallow at 0.93 or 1.50% calcium (Smith et al., 2003).

Table 4 summarizes the effects of the experimental diets on bone characteristics. As compared to the basal diet, feeding sunflower oil decreased dry tibia weight more than feeding tallow (5.5 vs. 5.9 g; $p<0.05$). Vitamin D supplementation tended to increase dry tibia weight ($p<0.07$). This trend however was not fat source dependent. None of the dietary factors affected tibia ash weight. Similarly, as compared to the basal diet, feeding sunflower oil tended decrease the amount of tibia ash weight more than feeding tallow (2.44 vs. 2.58 g; $p<0.09$). Except for vitamin D level, other dietary factors did not influence tibia ash percentage. Vitamin D supplementation reduced ash percentage in tibia (44.8 vs. 43.2%, $p<0.04$). Despite not being measured, this may suggest that bone Ca reserves might have been mobilized to support eggshell formation. In agreement with the present study, Smith et al. (2003) showed a decrease in tibia calcium level in broilers in response to fat feeding. However, increasing dietary calcium concentration did not ameliorate bone calcium concentration and retention. Without fat supplementation, Swiatkiewicz and Koreleski (2005) reported that simultaneous addition of particulate limestone and 25-OH vitamin D_3 improved loading capacity and bone stiffness. In another experiment, Koreleski and Swiatkiewicz (2005) reported that the interaction effect of limestone level by limestone source by vitamin D level was age dependent, with old laying hens being most responsive. Other studies involving phytase and vitamin D supplementation failed to demonstrate the beneficial effects of vitamin D supplementation on bone characteristics (Keshavarz, 2003; Puzio et al., 2004). Fritts and Waldroup (2003) increased dietary vitamin D concentration up to 4,000 IU per kilogram and reported improvement in bone breaking strength, increase in bone ash percentage, and decrease in tibial dyschondroplasia occurrence, with greatest response at 2,000 IU per kilogram. This beneficial effect is shown to be dose dependent, regardless of vitamin D source (Atencio et al., 2005; Mattile et al., 2004). In other experiment involving chicks housed at two stocking densities (680 and 340 cm^2/chick) and two levels of vitamin D supplementation (400 and 4,000 ICU/kg), it was shown that high density and excess vitamin D_3 resulted in an increase in the incidence of twisted leg (Cruickshank and Sim, 1987).

Egg quality and yolk fatty acid composition

Changes in inner and outer egg quality parameters in response to the dietary factors are shown in Table 5. Except

Table 5. The effects of additional vitamin D in the diets of peak producing hens fed different sources and levels of fat on physical egg quality

Dietary factors[1]			Response variables						
Fat source	Fat level	Vitamin D level	Shape index (%)	Shell strength (kg/cm^2)	Shell thickness ($mm*10^{-2}$)	Yolk color	Yolk index (%)	Albumen index (%)	Haugh unit
No fat	0%	1×	74.08	1.223	0.123	9.39	39.25	8.71	80.86
		3×	74.45	1.470	0.122	9.83	40.14	8.68	82.14
Sunflower oil	2.5%	1×	74.90	1.365	0.121	9.65	39.92	9.36	84.86
		3×	74.17	1.439	0.129	8.94	39.25	8.84	83.19
	5.0%	1×	73.64	1.414	0.123	10.22	40.31	9.14	82.88
		3×	74.75	1.469	0.119	9.10	40.91	9.51	85.61
Tallow	2.5%	1×	75.13	1.739	0.124	10.19	41.36	9.17	83.65
		3×	74.75	1.506	0.123	9.83	39.74	8.41	80.95
	5.0%	1×	72.52	1.323	0.121	10.09	39.82	8.78	83.32
		3×	75.22	1.510	0.123	9.83	41.09	8.44	81.06
SEM			0.58	0.125	0.002	0.23	0.56	0.43	1.73
ANOVA			---------- p<F ----------						
Fat source			0.82	0.28	0.94	0.003	0.28	0.15	0.21
Fat level			0.11	0.37	0.11	0.34	0.26	0.99	0.98
Linear effect of sunflower oil			0.84	0.45	0.64	0.71	0.13	0.22	0.19
Quadratic effect of sunflower oil			0.60	0.88	0.14	0.12	0.26	0.88	0.56
Linear effect of tallow			0.70	0.48	0.97	0.16	0.16	0.78	0.80
Quadratic effect of tallow			0.16	0.04	0.44	0.26	0.41	0.66	0.67
Vitamin D level			0.13	0.31	0.58	0.06	0.65	0.47	0.84
Fat source×fat level			0.40	0.20	0.37	0.20	0.19	0.49	0.80
Fat source×vitamin D level			0.28	0.64	0.64	0.07	0.84	0.45	0.27
Fat level×vitamin D level			0.15	0.28	0.12	0.66	0.10	0.32	0.37
Fat source×fat level×vitamin D level			0.44	0.25	0.02	0.44	0.32	0.82	0.60

[1] The experimental diets were basal diet not containing fat and basal diet plus 2.5 and 5.0% either sunflower oil or tallow with meeting (1×) and exceeding NRC recommendation (NRC, 1994) by 3-fold (3×).

for eggshell thickness, other quality parameters fluctuated as the experiment advanced (time effect, $p<0.0001$). None of the dietary factors affected shape index, eggshell strength, eggshell thickness, yolk index, albumen index, and Haugh unit. Eggshell strength quadratically increased with increasing tallow level ($p<0.04$). Yolk color for hens fed sunflower oil was lower than that for hens fed tallow (9.5 vs. 10.0; $p<0.003$). Vitamin D supplementation tended to decrease yolk color by 4% ($p<0.06$). This decrease tended to be at a greater magnitude when the diet contained sunflower oil compared to tallow ($p<0.07$). Information on responses of physical egg quality parameters to fat feeding is limited. Grobas et al. (2001) fed layers 0, 5, and 10% corn oil and tallow and reported no changes in physical egg quality parameters. Greater decline in eggshell strength in hens fed sunflower oil as compared to those fed tallow could be related to their influences on absorbability of dietary calcium. However, there was no fat type by vitamin D level interaction effect on eggshell strength. Keshavarz (2003), Mattila (2003), and Paik et al. (2005) supplemented laying hens with various levels of vitamin D and reported no effects on eggshell quality. Similarly, yolk color responses could be linked to availability of fat-soluble vitamins because fat feeding affects metabolism of fat-soluble nutrients (Mazalli et al., 2004).

Table 6 shows the effects of dietary factors on yolk fatty acid composition. None of the dietary factors affected $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$, $C_{20:4}$, $C_{22:2}$, and $C_{22:6}$ concentrations in egg yolk. Vitamin D supplementation caused decrease in $C_{18:0}$ from 17.6 to 17.0% ($p<0.06$). Moreover, supplemental vitamin D tended to decrease and increase $C_{18:1}$ concentration in yolk of hens fed sunflower oil and tallow, respectively ($p<0.08$). There was no fat source effect on yolk $C_{18:2}$ concentration. Increasing sunflower level quadratically decreased $C_{18:2}$ level in egg yolk ($p<0.04$). Vitamin D supplementation increased and decreased $C_{18:2}$ concentration in egg yolk of hens fed sunflower oil and tallow, respectively ($p<0.04$). There was no main effect of fat source on the $C_{22:5}$ level in egg yolk, but there was for fat level ($p<0.01$). Increasing sunflower oil level quadratically decreased yolk $C_{22:5}$ concentration ($p<0.005$). The $C_{22:5}$ level in egg yolk tended to be greater as the sunflower oil level increased from 2.5 to 5.0% than with increasing tallow from 2.5 to 5.0% ($p<0.07$). Nutritional implication of egg yolk has been of great concern for human nutrition. Thus, numerous studies have been employed to alter fatty acid composition of yolk (Baucells et al., 2000; Lim et al., 2006). Chickens have high lipogenic

Table 6. The effects of additional vitamin D in the diets of hens supplemented with different sources and levels of fat on yolk fatty acid composition

Dietary factors[1]			Fatty acids (% of total fatty acids)								
Fat source	Fat level	Vitamin D level	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{20:4}$	$C_{22:2}$	$C_{22:5}$	$C_{22:6}$
No fat	0%	1×	24.85	1.27	17.55	25.99	15.91	6.80	1.50	0.86	4.33
		3×	25.66	1.21	16.51	25.67	16.34	6.76	2.04	0.93	4.23
Sunflower oil	2.5%	1×	25.54	1.03	17.53	26.59	14.61	7.07	1.92	0.70	4.39
		3×	25.34	1.17	17.51	25.65	15.96	6.85	1.64	0.78	4.57
	5.0%	1×	24.73	1.17	17.92	26.91	15.63	6.22	1.31	0.99	4.27
		3×	25.50	1.03	17.04	25.27	16.08	6.95	1.55	0.97	4.59
Tallow	2.5%	1×	25.24	1.27	17.32	26.53	16.33	6.86	0.84	0.85	4.13
		3×	25.52	1.00	16.75	26.61	15.90	6.66	1.75	0.89	4.44
	5.0%	1×	25.72	1.07	17.45	25.38	15.94	6.67	2.21	0.84	4.59
		3×	25.85	1.27	17.37	26.68	15.53	6.60	1.14	0.98	4.51
SEM			0.43	0.13	0.45	0.82	0.44	0.25	0.64	0.07	0.18
ANOVA			---------- p<F ----------								
Fat source			0.33	0.61	0.40	0.74	0.27	0.67	0.79	0.56	0.77
Fat level			0.90	0.86	0.61	0.63	0.76	0.16	0.98	0.01	0.40
Linear effect of sunflower oil			0.72	0.20	0.33	0.88	0.56	0.63	0.56	0.33	0.31
Quadratic effect of sunflower oil			0.59	0.52	0.36	0.67	0.04	0.23	0.77	0.005	0.50
Linear effect of tallow			0.37	0.62	0.32	0.88	0.36	0.64	0.91	0.99	0.15
Quadratic effect of tallow			0.64	0.60	0.73	0.34	0.66	0.82	0.35	0.60	0.38
Vitamin D level			0.16	0.72	0.06	0.57	0.32	0.84	0.77	0.19	0.41
Fat source×fat level			0.25	0.83	0.52	0.67	0.14	0.48	0.43	0.07	0.23
Fat source×vitamin D level			0.91	0.86	0.85	0.08	0.04	0.28	0.95	0.58	0.61
Fat level×vitamin D level			0.51	0.65	0.78	0.82	0.50	0.13	0.43	0.97	0.62
Fat source×fat level×vitamin D level			0.37	0.06	0.31	0.42	0.47	0.25	0.18	0.37	0.31

[1] The experimental diets were basal diet not containing fat and basal diet plus 2.5 and 5.0% either sunflower oil or tallow with meeting (1×) and exceeding NRC recommendation (NRC, 1994) by 3-fold (3×).

activity and responsive to dietary fatty acid composition via altering nutrient partitioning (Newman et al., 2002). Increasing dietary fat level decreases saturated fatty acids in yolk. Increasing $C_{18:2}$ caused linear decreases in longer chain fatty acids (Grobas et al., 2001). In this study, fat feeding effects on egg yolk fatty acid composition were inconsistent with fatty acid composition of fats and known fates of fatty acid metabolism in chickens (NRC, 1994). Vitamin D addition however seems to interact with fat type.

CONCLUSIONS

In this experiment, the effects of different levels of sunflower oil and tallow in the diets meeting and exceeding vitamin D requirement by 3-fold on laying performance, metabolic profile, and egg quality were investigated during the peak laying period. Fat feeding increased body weight, decreased feed intake, did not alter egg weight, and improved feed conversion efficiency. Vitamin D supplementation had minor effects on laying performance responses. Lipid metabolites were responsive to fat feeding. Serum vitamin D concentrations were affected by dietary vitamin D level, but not fat type and level. Serum mineral concentration was independent from the dietary treatments. Fat feeding adversely affected tibia weight and vitamin D level ameliorated decline in tibia weight independently from the fat type. Fat feeding had minor effect on outer and inner egg quality parameters and yolk egg composition. Vitamin D effect on egg quality variables was negligible. This study failed to fully demonstrate that fat feeding depresses calcium metabolism and that hens fed fat demand more vitamin D.

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