# Research Note: Effect of increasing fat supplementation in diets on productive performance, egg quality, and fatty liver incidence in laying hens throughout the entire laying cycle

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**ABSTRACT** This study aimed to investigate the effect of increasing fat supplementation in diets on productive performance, egg quality, and fatty liver incidence in laying hens during the entire laying cycle. A total of three hundred eighty-four 18-wk-old Hy-Line Brown laying hens were randomly allotted to 1 of 3 dietary treatments with 8 replicates for a 52-wk feeding trial. Each replicate comprised 16 consecutive cages with 1 hen per cage. The experimental diets were prepared by supplementing 0, 1.5, or 3.0% tallow to a basal diet, but all nutrients and energy in 3 diets were formulated to be equalized according to the recommended nutrient and energy concentrations at each phase of laying hens. Results indicated that increasing fat supplementation in diets decreased (linear, P < 0.01) feed conversion ratio (**FCR**) by increased egg mass (linear, P < 0.05) but decreased feed intake (linear and quadratic, P < 0.05) in laying hens during overall periods. Increasing fat supplementation in diets decreased (linear and quadratic, P < 0.05) egg yolk color during overall periods. Increasing fat supplementation in diets had no effects on liver color and hemorrhagic score measured at 60 wk (phase 3) and 70 wk of age (phase 4) without affecting hepatic fat concentrations during overall periods. However, the relative abdominal fat weight in laying hens was increased (linear and quadratic, P <0.05) during overall periods by increasing fat supplementation in diets. In conclusion, increasing fat supplementation up to 3.0% in diets improves FCR with no impacts on fatty liver incidence and economics in laying hens throughout the entire laying cycle.

Key words: dietary fat, entire laying cycle, fatty liver incidence, laying hen, productive performance

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# INTRODUCTION

Most laying hens in the current production system are typically raised in conventional cage-type facilities throughout their entire laying cycle. This practice is known to cause various health problems, including fatty liver syndrome (**FLS**) or fatty liver hemorrhagic syndrome (**FLHS**). These metabolic diseases are frequently observed in caged laying hens, possibly due to excessive supply of dietary energy and nutrients, coupled with restricted physical activity (Cherian and Hayat, 2009). The accumulation of excessive fat in the liver leads to a structural and functional defect, resulting in increased hepatic diseases and eventual death (Shini et al., 2019). Therefore, it is crucial to prevent the occurrence of FLS or FLHS by reducing hepatic fat accumulation to

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improve productive performance and health in caged laying hens.

Dietary fat is commonly used to increase energy concentrations in animal diets. Furthermore, dietary fat supplementation has been shown to enhance feed palatability (Classen, 2017) and improve feed efficiency by promoting better nutrient utilization (Kim et al., 2019). However, despite these benefits, the use of dietary fat in laying hens is often limited because of concerns about increased feed costs. Nevertheless, if additional health and nutritional benefits of dietary fat are identified, fat supplementation in diets may be encouraged. Interestingly, increasing fat concentrations in diets have been reported to decrease hepatic lipogenesis in laying hens and rats through possible feed-back mechanisms (Haghighi-Rad and Polin, 1982; Zhang et al., 2014; Han et al., 2023). This leads to the hypothesis that increasing fat supplementation in diets can decrease the development of fatty liver in laying hens by decreasing de novo lipogenesis in the liver. However, information regarding the long-term effect of feeding diets supplemented with additional fat on productive performance, egg quality,

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and fatty liver incidence in laying hens is currently lacking.

Therefore, the objective of this study was to investigate the effect of increasing fat supplementation in diets on productive performance, egg quality, and fatty liver incidence in laying hens throughout the entire laying cycle.

# MATERIALS AND METHODS

### Birds, Diets, and Experimental Design

All experimental procedures were reviewed and approved by the Animal Care and the Use Committee at Chung-Ang University. A total of 384 Hy-Line Brown laying hens at 18 wk of age with the similar BW (1.42  $\pm$ 0.01 kg) were allotted to 1 of 3 dietary treatments with 8 replicates in a completely randomized design. Each replicate had 16 consecutive cages with 1 hen per cage  $(24 \times 36 \times 39 \text{ cm})$ . All hens were fed a commercial prelaying diet before the start of experiment. A conventional 4-phase-feeding program was adopted in the current study during a 52-wk feeding trial from 18 to 70 wk of age (i.e., phase 1: 18-34 wk; phase 2: 35-50 wk; phase 3:51-62 wk; phase 4:63-70 wk) according to the management guideline of Hy-Line Brown International (2018) with minor modifications. The basal diet (CON) was formulated to contain the recommended concentrations of energy and nutrients for each phase of the laying period (Hy-Line Brown International, 2018) without fat supplementation (Table 1). Two additional diets were prepared by supplementing 1.5% (T1.5) and 3.0% tallow (T3.0) to the basal diet. The commercial tallow, the most widely used in layer industries, was used as a supplemental fat source. The calculated concentrations of energy and nutrients in all diets were equalized at each laying phase by adjusting inclusion levels of other nonfat ingredients. The analyzed fat concentrations in treatment diets are presented in Table 1. Hens were housed in 3-tier battery cages with free access to water, whereas weekly FI was controlled according to the management guideline of Hy-Line Brown International (2018) to prevent early reproductive disorders and excessive BW. All hens were raised under a programmed lighting (16L:8D) and received a periodic ventilation throughout the experiment.

# Productive Performance and Egg Quality

Productive performance including hen-day egg production and egg weight was recorded daily during the experiment. However, the productive performance recorded from 22 wk of age were used for the data analysis because the beginning time of the first egg was 21 wk of age and the number of eggs before 22 wk of age was only 9. Feed intake (**FI**) and BW were calculated based on a replicate every 4 wk. Total egg production (**TEP**) was determined based on a replicate every week by adding the number of all eggs measured by an egg grading machine (Yuhan Machinery Co., Daejeon, Republic of Korea). Egg quality was assessed with randomly collecting samples of 15 eggs per replicate, with 5 eggs per d at 4wk intervals. Eggshell color was measured using an eggshell color fan (Samyangsa, Kangwon-do, Republic of Korea). Eggshell strength, egg yolk color, and Haugh unit were evaluated using a digital egg tester (DET-6000, Nabel Co., Ltd., Kyoto, Japan) based on the method reported previously (Han et al., 2023). All data for productive performance and egg quality were summarized for each phase of the laying period.

The return over feed cost (\$/hen) was calculated by subtracting feed costs from egg incomes (dePersio et al., 2015). The egg income was calculated from the eggs obtained by hens and the price of eggs by size and the feed cost was calculated from the amount of FI and the price of the feed. All calculations were made using South Korean currency won (**KRW**). The resulting values were converted to United States dollars (**USD**) with an exchange rate of USD 1.00 = KRW 1,200 (Koiyama et al., 2018).

## Sample Collection

One hen per replicate with a BW close to the average BW of each replicate (i.e., 8 birds per treatment) was selected. The selection was conducted at the middle or end of each phase (i.e., 30, 46, 60, and 70 wk of age for phase 1, 2, 3, and 4, respectively). The selected hens were euthanized using  $CO_2$  asphyxiation and promptly dissected.

The serum samples were collected and used to diagnose the FLHS in the end of the experiment (i.e., 70 wk of age; Zhang et al., 2008). The serum concentrations of aspartate aminotransferase (**AST**), alanine aminotransferase (**ALT**), total cholesterol, lactate dehydrogenase (**LDH**), and triglyceride (**TG**) were analyzed using a HITACHI 7020 automatic analyzer (Hitachi, Tokyo, Japan).

A small portion of the liver was collected for the analysis of total fat concentrations by acid-hydrolyzed ether extraction (method 996.01; AOAC, 2007). The abdominal fat pads were collected and weighed to determine its relative weight as a percentage of total BW (Han et al., 2023). In addition, fatty liver incidence was determined based on the liver color and hemorrhagic score following the previously established method (Han et al., 2023). Briefly, the liver was photographed and rated on a scale from 1 to 5 (1 = dark red; 5 = yellowish red). The liver hemorrhagic score was also recorded on a scale from 0 to 5 (0 = normal liver; 5 = large and massive hemorrhages). However, liver color score and liver hemorrhagic score were only measured in 60 wk (phase 3) and 70 wk of age (phase 4).

### Statistical Analysis

All data were analyzed by ANOVA as a completely randomized design using the PROC MIXED procedure (SAS Institute Inc., Cary, NC). Each replicate was

Items	Phase 1 $(18-34 \text{ wk})$			Phase 2 (35–50 wk)			Phase 3 (51-62 wk)			Phase 4 $(63-70 \text{ wk})$		
	CON	T1.5	T3.0	CON	T1.5	T3.0	CON	T1.5	T3.0	CON	T1.5	T3.0
Ingredients (%)												
Corn	63.97	61.00	58.00	65.39	62.40	59.24	64.88	61.14	57.46	64.27	60.30	56.33
Soybean meal, 46% CP	11.24	16.30	21.41	15.93	20.82	25.02	13.03	16.62	20.56	13.00	16.14	19.32
Corn gluten meal	10.93	7.70	4.44	5.80	2.67	0.06	5.32	2.97	0.37	4.58	2.50	0.40
Wheat bran							3.00	4.00	5.00	4.00	5.30	6.60
Tallow		1.50	3.00		1.50	3.00		1.50	3.00		1.50	3.00
Monodicalcium phosphate	1.85	1.82	1.79	1.48	1.47	1.44	1.32	1.27	1.24	1.23	1.19	1.15
Limestone	10.05	10.02	9.98	9.70	9.66	9.62	10.29	10.28	10.25	11.15	11.13	11.12
54% L-lysine H <sub>2</sub> SO <sub>4</sub>	0.62	0.44	0.26	0.32	0.15	0.00	0.40	0.27	0.12	0.36	0.24	0.12
98.5% L-Threonine	0.11	0.08	0.05	0.05	0.03	0.01	0.08	0.06	0.04	0.08	0.07	0.05
Liquid DL-methionine	0.23	0.24	0.26	0.21	0.22	0.23	0.23	0.24	0.25	0.20	0.21	0.22
L-tryptophan	0.31	0.21	0.12	0.15	0.07	0.00	0.17	0.10	0.03	0.12	0.07	0.02
Celite				0.28	0.32	0.69	0.59	0.86	0.99	0.32	0.66	0.98
Premix <sup>2</sup>	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated energy and nutrient con	centration											
$\mathrm{AME}_\mathrm{n},\mathrm{kcal/kg}$	2,860	2,860	2,860	2,800	2,800	2,800	2,770	2,770	2,770	2,740	2,740	2,740
CP, %	17.05	17.05	17.05	15.92	15.92	15.92	14.79	14.79	14.79	14.40	14.40	14.40
SID lysine, %	0.81	0.81	0.81	0.73	0.73	0.73	0.71	0.71	0.71	0.69	0.69	0.69
$\mathrm{SID}\ \mathrm{methionine} + \mathrm{cysteine}, \%$	0.69	0.69	0.69	0.63	0.63	0.63	0.62	0.62	0.62	0.59	0.59	0.59
SID threenine, %	0.57	0.57	0.57	0.52	0.52	0.52	0.50	0.50	0.50	0.50	0.50	0.50
SID tryptophan, %	0.18	0.18	0.18	0.17	0.17	0.17	0.16	0.16	0.16	0.15	0.15	0.15
Total calcium, %	4.08	4.08	4.08	3.91	3.91	3.91	4.09	4.09	4.09	4.40	4.40	4.40
Available phosphorus, %	0.45	0.45	0.45	0.38	0.38	0.38	0.35	0.35	0.35	0.33	0.33	0.33
Crude fat, $\%^3$	2.24	4.13	5.20	2.37	4.37	5.91	2.94	4.39	6.43	2.96	4.44	6.46

**Table 1.** Composition and nutrient concentrations of experimental diets throughout the entire laying cycle.<sup>1</sup>

 $^{1}$ CON = no supplementation with tallow; T1.5 = basal diet + 1.5% tallow; T3.0 = basal diet + 3.0% tallow.

<sup>2</sup>Provided per kg of the complete diet: vitamin A, 10,000 IU (retinyl acetate); vitamin D3, 4,500 IU; vitamin K3, 3.0 mg (menadione dimethylpyrimidine); vitamin B1, 2.50 mg; vitamin B2, 6.50 mg; vitamin B6, 3.20 mg; vitamin B12, 18.0  $\mu$ g; biotin, 180  $\mu$ g; biotin, 20 mg; vitamin B1, 2.50 mg; vitamin B2, 6.50 mg; vitamin B6, 3.20 mg; vitamin B12, 18.0  $\mu$ g; biotin, 180  $\mu$ g; biotin, 190  $\mu$ g; biotin, 180  $\mu$ g; biotin, 190  $\mu$ g; biotin, 19

<sup>3</sup>Analyzed crude fat concentrations in diets (method 960.39; AOAC, 2007).

considered an experimental unit. All data were checked for normal distribution and outliers using the UNIVAR-IATE procedure of SAS. The LSMEANS procedure was used to calculate the treatment means and the PDIFF option was used to separate the means within each phase. The orthogonal polynomial contrast test was also used to determine linear and quadratic effects of increasing fat supplementation in diets. The significance level was set at P < 0.05.

# **RESULTS AND DISCUSSION**

Hen-day egg production was not affected by increasing fat supplementation in diets for individual phases and overall phase, although a quadratic association (P < 0.05) was observed in phase 1 (Table 2). Increasing fat supplementation in diets did not affect TEP for individual phases and overall phase, although a linear and quadratic association (P < 0.05) was observed in phase 1. However, egg weight was increased (linear, P < 0.01) by increasing fat supplementation in diets for individual phases and overall phase, except for phase 4. Similarly, egg mass was increased (linear, P < 0.05) by increasing fat supplementation in diets for phase 3 and overall phase. Increasing fat supplementation in diets decreased FI (linear and quadratic, P < 0.05) for phase 1 and overall phase. Therefore, the increase in egg mass coupled with the decrease in FI resulted in a decrease (linear, P< 0.01) in FCR for phase 1, 3, and overall phase. The BW was increased (linear, P < 0.01) by increasing fat supplementation in diets for individual phases and overall phase, except for phase 1 exhibiting a quadratic relationship (P < 0.05).

Table 2. Effect of increasing fat supplementation in diets on productive performance and egg quality in laying hens throughout the entire laying cycle.<sup>1,2</sup>

	Supp	lemental levels of tallo	w (%)		P-value <sup>3</sup>		
Item	0	1.5	3.0	SEM	Т	$\mathbf{L}$	$\mathbf{Q}$
Productive perform							
Hen-day egg pro	duction (%)						
Phase 1	80.7	82.7	79.9	0.98	0.114	0.573	0.044
Phase 2	93.0	92.3	91.7	0.48	0.184	0.070	0.930
Phase 3	91.0	92.0	92.2	0.79	0.486	0.285	0.624
Phase 4	89.5	90.7	91.1	0.95	0.446	0.232	0.728
Overall	88.5	89.4	88.7	0.61	0.508	0.822	0.263
TEP (count/rep							
Phase 1	984 <sup>a</sup>	$1,008^{a}$	$934^{\mathrm{b}}$	12.7	0.002	0.011	0.00
Phase 2	1,451	1,449	1,436	14.0	0.705	0.458	0.714
Phase 3	919	936	936	15.7	0.684	0.458	0.658
Phase 4	519	535	532	12.4	0.635	0.472	0.537
Overall	3,872	3,927	3,837	37.8	0.256	0.515	0.131
EW (g)	0,012	0,021	0,001	01.0	0.200	0.010	0.10
Phase 1	$58.4^{\mathrm{b}}$	$59.0^{\mathrm{ab}}$	$59.5^{\mathrm{a}}$	0.26	0.020	0.006	0.881
Phase 2	$62.1^{\rm b}$	$62.7^{\rm b}$	63.6 <sup>a</sup>	0.32	0.020	0.003	0.638
Phase 3	$63.9^{b}$	$64.6^{\mathrm{ab}}$	65.2 <sup>a</sup>	0.32	0.011	0.005	0.038
Phase 3 Phase 4	64.3			0.32	0.022		
	$64.3 \\ 62.2^{b}$	$64.4 \\ 62.7^{ab}$	65.0			0.210	0.740
Overall	62.2	62.7	$63.3^{\mathrm{a}}$	0.28	0.025	0.007	0.833
EM (g)	17.1	10.0	17 0	0.01	0.101	0.410	0.05
Phase 1	47.1	48.8	47.6	0.61	0.121	0.612	0.050
Phase 2	57.7	57.8	58.3	0.42	0.592	0.340	0.705
Phase 3	$58.1^{\mathrm{b}}$	$59.4^{a}$	$60.0^{\mathrm{a}}$	0.43	0.010	0.003	0.549
Phase 4	57.5	58.5	59.2	0.62	0.169	0.064	0.874
Overall	55.1	56.1	56.3	0.38	0.071	0.038	0.346
${ m FI}~{ m (g/hen/d)}$			1				
Phase 1	$100.7^{a}$	101.1 <sup>a</sup>	$97.0^{\mathrm{b}}$	0.57	< 0.001	0.001	0.003
Phase 2	106.9	106.1	106.2	0.39	0.260	0.214	0.302
Phase 3	109.9	109.8	109.9	0.03	0.628	0.550	0.468
Phase 4	109.8	109.9	109.8	0.04	0.884	0.786	0.675
Overall	$106.8^{a}$	$106.7^{\rm a}$	$105.7^{\rm b}$	0.13	< 0.001	< 0.001	0.010
FCR (g/g)							
Phase 1	$2.14^{\mathrm{a}}$	$2.07^{\mathrm{b}}$	$2.04^{b}$	0.024	0.020	0.007	0.491
Phase 2	1.85	1.84	1.82	0.013	0.267	0.111	0.890
Phase 3	$1.89^{\mathrm{a}}$	$1.85^{b}$	$1.83^{b}$	0.014	0.010	0.003	0.527
Phase 4	1.91	1.88	1.86	0.021	0.176	0.067	0.871
Overall	1.95 <sup>a</sup>	1.91 <sup>b</sup>	1.89 <sup>b</sup>	0.013	0.006	0.002	0.542
BW (kg)	1.00	1.01	1.00	01010	0.000	01002	0.01
Phase 1	1.86	1.87	1.87	0.004	0.062	0.222	0.040
Phase 2	$1.97^{\rm b}$	2.01 <sup>a</sup>	2.03 <sup>a</sup>	0.009	<0.002	<0.001	0.602
Phase 3	$2.04^{\rm b}$	2.01 <sup>a</sup>	2.12 <sup>a</sup>	0.005	< 0.001	<0.001	0.318
Phase 4	$2.04^{\circ}$	$2.03^{a}$	2.12 $2.16^{a}$	0.012	0.001	0.001	0.488
Overall	$1.99^{\rm b}$	2.13 $2.03^{a}$	2.10 $2.04^{a}$	0.015	<0.004	<0.001	0.480
Egg quality	1.99	2.05	2.04	0.009	<0.001	<0.001	0.50.
Eggshell strength	$(lra/am^2)$						
		9.75	9.70	0.000	0.000	0.000	0.01
Phase 1	3.74	3.75	3.76	0.029	0.882	0.629	0.914
Phase 2	3.84	3.86	3.92	0.047	0.448	0.226	0.741

(continued)

#### Table 2 (Continued)

Item	Supple	emental levels of tallow	v (%)	SEM	$P ext{-value}^3$			
	0	1.5	3.0		Т	L	Q	
Phase 3	3.94	4.01	3.95	0.078	0.771	0.952	0.478	
Phase 4	3.64	3.67	3.64	0.067	0.939	0.969	0.728	
Overall	3.79	3.82	3.82	0.038	0.804	0.603	0.692	
Egg yolk color								
Phase 1	$10.5^{a}$	$10.0^{b}$	9.3 <sup>°</sup>	0.07	< 0.001	< 0.001	0.235	
Phase 2	$8.2^{\mathrm{a}}$	$7.1^{\mathbf{b}}$	$5.7^{c}$	0.07	< 0.001	< 0.001	0.041	
Phase 3	$8.3^{\mathrm{a}}$	$7.2^{\mathbf{b}}$	$5.9^{\circ}$	0.06	< 0.001	< 0.001	0.023	
Phase 4	8.3 <sup>a</sup>	$7.6^{\mathbf{b}}$	$6.7^{\circ}$	0.05	< 0.001	< 0.001	0.552	
Overall	$8.8^{\mathrm{a}}$	$8.0^{\mathbf{b}}$	$6.9^{\circ}$	0.05	< 0.001	< 0.001	0.032	
Haugh unit								
Phase 1	99.0	98.2	98.2	0.294	0.115	0.072	0.286	
Phase 2	94.2	94.4	94.0	0.513	0.832	0.770	0.601	
Phase 3	90.4	91.1	91.1	0.526	0.577	0.356	0.630	
Phase 4	88.7	89.1	89.0	1.034	0.953	0.835	0.823	
Overall	93.1	93.2	93.1	0.481	0.974	0.998	0.821	

Abbreviations: BW, body weight; EM, egg mass; EW, egg weight; FCR, feed conversion ratio; FI, feed intake; TEP, total egg production.

<sup>a-c</sup>Means in the same row with different superscripts are different (P < 0.05).

<sup>1</sup>Data are least squares means of 8 observations per treatment.

<sup>2</sup>A phase-feeding program was used during the experiment from 22 to 70 wk of age (phase 1: 22-34 wk; phase 2: 35-50 wk; phase 3: 51-62 wk; phase 4: 63-70 wk).

<sup>3</sup>T, overall effect of treatments; L, linear effect of increasing supplementation of fat in diets; Q, quadratic effect of increasing supplementation of fat in diets.

 ${}^{4}\text{TEP}$  was calculated by adding the number of all eggs from each phase, which means the total egg number per replicate.

The observation of decreased FCR by increasing fat supplementation in diets throughout the entire laying cycle was attributed to decreased FI and increased egg weight in the current study. Dietary fat is known to have an extra-caloric effect by providing additional available energy to animals because dietary fat induces lower heat increment, resulting in increased available energy supply (Summers, 1984). Moreover, dietary fat improves nutrient utilization in diets by increasing the retention time of digesta in the intestine (Summers, 1984). Therefore, decrease in FI due to dietary fat supplementation may be caused by increased available energy supply because hens tend to decrease FI in response to increased available energy supply (Classen, 2017). However, it is important to note that energy concentrations expressed as AME<sub>n</sub> were equalized in the current treatment diets across all phases, indicating that energy value of dietary fat expressed in AME<sub>n</sub> may be underestimated. This may highlight the importance of formulating animal diets based on NE systems rather than ME systems (Kil et al., 2013). However, the information for NE system in poultry diets is largely lacking.

Furthermore, the increase in egg weight by increasing fat supplementation is also likely attributed to improved nutrient supply in addition to increased available energy. Increased energy and nutrient supply may also explain the increased BW in this study. Additionally, hens with higher BW generally exhibit higher egg weight (Summers and Leeson, 1983), indicating that increased egg weight may also be a result of increased BW in this study.

Egg quality including eggshell strength, Haugh unit, and eggshell color (data not shown) was not affected by increasing fat supplementation in diets for all laying phases (Table 2). However, increasing fat supplementation in diets consistently decreased (linear, P < 0.01) egg yolk color for all laying phases. This observation is primarily due to increased substitution of corn grains and corn gluten meal, which contain high amounts of xanthophyll contributing to egg yolk color, with increasing fat supplementation in order to equalize energy concentrations among treatment diets. Previous studies have reported that decreasing inclusion of corn or corn byproducts in diets decreased egg yolk color in laying hens (Kim et al., 2019; Han et al., 2023).

Liver color score and liver hemorrhagic score, measured at 60 wk of age (phase 3) and 70 wk of age (phase 4), did not differ among dietary treatments (Figure 1A and B). Likewise, hepatic fat concentrations were not affected by increasing fat supplementation in diets for overall phase (Figure 1C). Interestingly, increasing fat supplementation in diets decreased (linear, P < 0.05) hepatic fat concentrations for phase 2, whereas it increased (linear, P < 0.05) hepatic fat concentrations for phase 3. However, the relative weight of abdominal fat to BW was increased by increasing fat supplementation in diets for phase 1 (linear, P < 0.05), phase 4, and overall phase (linear and quadratic, P < 0.05; Figure 1D).

The absence of effects of increasing fat supplementation on liver appearance and fat concentrations as indicators of fatty liver incidence may suggest that longterm feeding of diets supplemented with 1.5 or 3.0% fat has little positive impacts on decreasing fat accumulation in the liver of laying hens throughout the entire laying cycle. Likewise, in the present study, all serum measurements (e.g., AST, ALT, total cholesterol, LDH, and TG), which are related to hepatic TG accumulation and FLHS (Zhang et al., 2008), at the end of experiment were not affected by increasing fat supplementation in diets (data not shown). Therefore, our hypothesis that additional fat supplementation decreases de novo

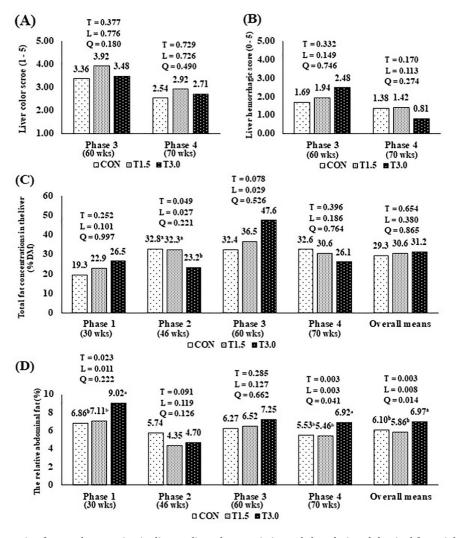


Figure 1. Effect of increasing fat supplementation in diets on liver characteristics and the relative abdominal fat weight in laying hens throughout the entire laying cycle. (A) Liver color score. (B) Liver hemorrhagic score. (C) Total fat concentrations in the liver (% DM). (D) The relative abdominal fat weight to BW (%). Abbreviations: CON, no supplementation with tallow; L, linear effect of increasing supplementation of fat in diets; Q, quadratic effect of increasing supplementation of fat in diets; T, overall effect of treatments; T1.5, basal diet + 1.5% tallow; T3.0, basal diet + 3.0% tallow.

lipogenesis in the liver by feed-back mechanisms was not verified. However, it can be speculated that the lack of further increase in hepatic fat accumulation by increasing fat supplementation in diets may indirectly indicate a decrease in hepatic lipogenesis because the liver is the main organ for de novo lipogenesis in poultry (Hermier, 1997). On the other hand, increased abdominal fat weight was observed by increasing fat supplementation in this study, suggesting that additional fat intake from increasing fat supplementation is subject to abdominal fat accumulation in laying hens. However, further studies are required to investigate how additional fat intake is transferred and utilized in the liver and abdominal fat of laying hens throughout the entire laying cycle.

In economic analysis, feed cost (\$/hen) was linearly increased with increasing fat supplementation in diets for overall periods (i.e., \$9.51, \$9.65, and \$9.79 for CON, T1.5, and T3.0, respectively). However, the return over feed cost (\$/hen) was not affected by increasing fat supplementation in diets for overall periods (i.e., \$15.38, \$15.55, and \$15.46 for CON, T1.5, and T3.0, respectively). Therefore, it is suggested that increasing fat supplementation in layer diets does not result in economic losses by increasing feed costs because of improved feed efficiency.

In conclusion, increasing fat supplementation up to 3.0% in diets improves FCR in laying hens by increased egg weight and decreased feed intake throughout the entire laying cycle. However, this long-term feeding of diets supplemented with fat increases abdominal fat accumulation without affecting fatty liver incidence in laying hens.

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# DISCLOSURES

The authors declare no conflict of interest for the data presented in this experiment.

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