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Combined effect of shortened photoperiod and low crude protein diet on liver triglyceride accumulation and lipid-related gene expression in quail

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ABSTRACT

A short photoperiod is reported to promote body weight gain in quail, and might therefore influence lipid metabolism and induce liver steatosis. Protein deficiency or malnutrition can also cause hepatic steatosis in mammals, but the effect in birds has not previously been investigated. In order to determine the influence of a shortened photoperiod and low crude protein diet on avian hepatic lipid metabolism, 10-day-old male Japanese quail (*Coturnix coturnix japonica*) were assigned to a 2 × 2 factorial arrangement of treatments in four groups, each of eight birds, and fed for three days, after which liver triglyceride (TG) accumulation and lipid-related gene expression were measured. Experimental factors included two dietary protein levels of 24% or 10% crude protein (CP), and two photoperiod conditions of a 12:12 h light/dark cycle (12L:12D) or a 1:23 h light/dark cycle (1L:23D). The liver TG level in quail fed the 10% CP diet under 1L:23D was higher than in birds maintained on either the 10% CP diet under 12L:12D (15.86 vs. 10.56 mg/g liver, $P < 0.01$) or 24% CP diet under 1L:23D (7.95 mg/g liver, $P < 0.01$). The sterol regulatory element-binding protein 1 mRNA, which is involved in TG synthesis, was more highly expressed in quail on the 10% CP diet under 1L:23D than in those maintained on the 24% CP diet under 12L:12D (2.2 vs. 7.9, $P < 0.01$), whereas the mRNA level of very low-density apolipoprotein II (*apoVLDLII*), which encodes a VLDL structural protein, was lower (157.3 vs. 12.5, $P < 0.01$). To our knowledge, this is the first report of a selective protein restriction and shorter photoperiod, alone and in combination, inducing a fatty liver state in an avian species. Our study provides insight into factors that govern lipid metabolism in birds, and the efficiency of domestic fowl production.

1. Introduction

Animals in the wild often face the possibility of starvation, and have evolved specific strategies to survive food shortages. For example, in mammals, including humans, a key adaptive response to a period of fasting is a shift from carbohydrates to ketone bodies for utilization as a primary carbon source. In this state, fatty acids (**FA**) are mobilized from adipose tissue to the liver and oxidized to acetyl-coenzyme A, which is a substrate for ketogenesis. Although the mechanisms are not fully understood, 12–36 h of fasting can increase liver triglyceride (**TG**) levels in mice (Badman et al., 2007; Sokolović et al., 2010) and humans (Moller et al., 2008). In contrast, birds have evolved different metabolic processes. Unlike mammals, plasma glucose in birds is maintained at a high level during fasting, and non-esterified free fatty acids (**NEFA**) supply the energy demands of the skeletal muscles (Sweazea and Braun, 2006; Saneyasu et al., 2013). There have been no reports of food deprivation leading to steatosis in birds; however, in waterfowl such as quail, liver steatosis can occur spontaneously due to energy storage before migration, or by overfeeding, which results in a product known as foie gras (Herault et al., 2010). Although the underlying metabolic changes are poorly understood, and despite only a few studies having been undertaken on this subject, birds can serve as a unique model in studying hepatic steatosis and lipid metabolism (Saadoun and Leclercq, 1987; McCue et al., 2013; Donaldson et al., 2017).

The Japanese quail (*Coturnix coturnix japonica*) has long been used in poultry production and has advantages over the chicken (*Gallus gallus*)—another Phasianidae family member—including resistance to certain poultry diseases, early onset of oviposition, and high production rates (Magubane et al., 2013). Furthermore, quail are also considered a good laboratory model because of their small size but robust physique,

Abbreviations: FA, fatty acids; TG, triglyceride; NEFA, non-esterified free fatty acids; VLDL, very low-density lipoprotein; CP, crude protein; *Fabp1*, fatty acid binding protein 1; *Srebp1*, sterol regulatory element-binding protein 1; *Ppara*, peroxisome proliferator-activated receptor α; *Cpt1*, carnitine palmitoyltransferase 1; *apoVLDLII*, very low-density apolipoprotein II; *RPS13*, ribosomal protein 13; Lb-FABP, liver basic type FABP; L-FABP, liver type FABP

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short generation time, and reproductive performance (Yoshimura et al., 2003; Li et al., 2012; Khosravi et al., 2016). Japanese quail are highly sensitive to photoperiod, and many physiological and behavioral changes—for instance, pituitary gland activity, gonad weight, and production of sex hormones such as androgen and progesterone—are increased by a prolonged photoperiod (Yoshimura et al., 2003; Satterlee et al., 2006). Conversely, increased body weight and delayed sexual maturation are observed when the photoperiod is shortened to less than four hours per day (Sakurai, 1980). Thus, decreasing the photoperiod length may also influence lipid metabolism and induce hepatic steatosis in quail. However, little attention has been given to the positive effects of a shortened photoperiod, especially with respect to lipid metabolism in birds, which could promote effective utilization of physiological resources and may consequently affect the efficiency of domestic fowl production.

In mammals, hepatic steatosis can result from increased uptake or intrahepatic synthesis of FAs, *de novo* lipogenesis, decreased rate of FA β-oxidation, and/or decreased synthesis or secretion of very low-density lipoprotein (**VLDL**) (Roden, 2006; Herault et al., 2010), protein deficiency, or malnutrition (Meghelli-Bouchenak et al., 1989; Zivkovic et al., 2007), possibly through upregulation of insulin signaling and inhibition of gluconeogenesis (Toyoshima et al., 2010; Yokota et al., 2014). Nutritional stress (starvation) affects the FA composition of TG in Japanese quail (Ben-Hamo et al., 2013) and, combined with lower ambient temperatures, causes a decrease in metabolic rate. Based on these studies, it was hypothesized that the combination of a shortened photoperiod and low crude protein (**CP**) intake could induce hepatic TG accumulation in quail.

To examine this hypothesis, we evaluated the effects of a shortened photoperiod and low CP diet on hepatic lipid metabolism in quail. Furthermore, we examined the quail liver to determine the expression of genes related to FA trafficking and metabolism (fatty acid binding protein 1 [***Fabp1***]), TG synthesis (sterol regulatory element-binding protein 1 [***Srebp1***]), β-oxidation of FAs (peroxisome proliferator-activated receptor α [***Pparα***], and carnitine palmitoyltransferase 1 [***Cpt1***]), and also a VLDL structural protein (very low-density apolipoprotein II [***apoVLDLII***]).

2. Materials and methods

2.1. Animals and diets

All animal experiments were performed according to the Regulations for Animal Care and Use of Waseda University, and were conducted with the permission of the Committee for Animal Experimentation of the School of Science and Engineering at Waseda University (approval number: 2013-A063, 2014-A019). Japanese quail (*Coturnix coturnix japonica*) were purchased at three days old from an animal breeding company (Motoki Corporation, Saitama, Japan). Ten-day-old male Japanese quail were housed at 37 ± 2 °C under a 12:12 h light/dark cycle (12L:12D) with light provided by white fluorescent lamps (100–200 lx), and provided with basic control food (24% CP diet) and water ad libitum before the start of the experiments. The quail were separated into four groups with other individuals of similar body weight (n = 8 per group). Two groups were fed a 24% CP diet and maintained under either 12L:12D or a short photoperiod of 1:23 h light/dark (1L:23D), and two groups were maintained on a low CP diet (10% CP diet), also under either 12L:12D or 1L:23D. The composition of the control and low CP diets is shown in Table 1.

2.2. Measurement of fatty acid levels

After three days, the quail were euthanized, and whole blood samples were collected by cardiac puncture using 1 mL plastic syringes without anticoagulant for plasma production. Simultaneously, the entire liver tissue was removed from individual birds and the wet weight of each measured, followed by rapid freezing in a deep freezer at − 80 °C. Hepatic TG levels were measured as previously described (Kudo et al., 2007; Fuse et al., 2012). In brief, blood samples were centrifuged to obtain plasma, which was then measured colorimetrically with enzymatic assay kits (Triglyceride E-Test and NEFA C-Test Wako; Wako Pure Chemical Industries Ltd., Osaka, Japan), according to the manufacturer's instructions.

Table 1
Semi-synthetic diet for quail (g/kg, air-dried weight).

Ingredients	Protein levels		Nutrients	Protein levels	
	Control	Low		Control	Low
Corn grain	530.0	780.0	Crude protein (%)*	24.35	10.21
Soybean meal	288.8	45.0	Crude fat (%)*	2.60	3.28
Corn gluten meal	75.5	0	ME (kcal)	2810	2820
Fish meal (65% CP)	27.8	0			
Alfalfa meal	0	100			
D-/L-methionine	1.4	0.8			
Lysine hydrochloride	0.2	1.6			
Threonine	3.3	1.5			
Isoleucine	1.0	1.0			
Arginine	0	0.8			
Cellulose	51.6	48.9			
Vitamin premix	2.0	2.0			
Mineral premix	18.4	18.4			

CP, crude protein; ME, metabolizable energy.
* Calculated values.

2.3. Gene expression analysis by real-time quantitative PCR (RT-PCR)

Changes in the expression of genes related to lipid metabolism were assessed by RT-PCR. A piece of liver tissue from each animal was dissolved in RNA-Solv Reagent (Omega Bio-Tek, Norcross, GA, USA), and total RNA was extracted and PCR performed as previously described (Kudo et al., 2007; Fuse et al., 2012). Gene-specific primer pairs (Table 2) were designed based on published data for the *Fabp1, Srebp1, Pparα, Cpt1*, and *apoVLDLII* genes (Herault et al., 2010; Yang et al., 2013). The relative level of each gene was normalized to that of ribosomal protein 13 ([***RPS13***]), a housekeeping gene in quail (Serr et al., 2011), and analyzed using the comparative Ct method (ΔΔCt method). A melt curve analysis revealed an absence of nonspecific products.

2.4. Data analysis

All data are expressed as means ± standard errors of the mean (SEM). Statistical analyses were performed using GraphPad prism (Ver. 6.07; GraphPad software, San Diego, CA, USA). After confirming a normal distribution using the Kolmogorov goodness-of-fit test, a two-way analysis of variance was performed; for P values < 0.05, a Šidák

Table 2
Primer sequences used for real-time PCR.

Gene	Forward (F) and Reverse (R) primes	Base pairs	Accession number
Fabp1	F-GCTGACAGGAGAGAAGGCCAA R-TGCAGGGTCTCTAGATTCTCTTGC	143	NM_001030731.1
Srebp1	F-CGGAAAGCCATCGAGTACATC R-CCATCCTCAGGCTGAGGTTCT	78	AY613441.1
Pparα	F-GCCTTTCAGTTGGAATGTCACATA R-CTGCCTTCAACTTGGCCTTCT	79	AF481797.1
Cpt1	F-TGAACACGGCAAACTTTCTG R-ATTCATAAGTGGCCGGACTG	172	NM_001012898.1
apoVLDLII	F-CAGTTCTTGCTGGATGTTTCCCAGAC R-CAATGGCCAAGTCATTCAGGAGGA	430	S82591.1
Rps13	F-AAGAAGGCTGTTGCTGTTCG R-GGCAGAAGCTGTCGATGATT	168	NM_001001783.1