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Feed restriction ameliorates metabolic dysregulation and improves reproductive performance of meat-type country chickens☆

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ABSTRACT

Restricted feed intake improves egg production in Cornish × Plymouth Rock (broiler) hens. Red-feather (RF) and Black-feather (BF) chickens are 2 local strains of non-broiler meat-type chickens whose egg production has declined with continued selection for meat yield, and which are unstudied regarding restricted feeding and egg-laying improvement. Sixteen week old RF and BF pullets were either fed *ad libitum* (AL) or restricted to 85% AL intake (R). At 35 wk and 50 wk R-hens showed improved egg production and less abnormal ovarian morphology than AL-hens. Obesity, hepatic steatosis, lipotoxic change to plasma lipids, and systemic inflammation induced by AL feeding in RF and BF hens were similar to those observed previously in AL-broiler hens. Egg production was negatively correlated to body weight, fractional abdominal fat weight and plasma NEFA concentrations in AL hens ($P < 0.05$). AL-hen hierarchical follicles accumulated ceramide and increased interleukin-1β production ($P < 0.05$) in conjunction with increased granulosa cell apoptosis, follicle atresia, ovarian regression, and reduced plasma 17β-estradiol concentrations ($P < 0.05$). The present outcomes from non-broiler but nevertheless meat-type country chicken strains indicate that selection for rapid growth and increased meat yield fundamentally changes energy metabolism in a way that renders hens highly susceptible to reproductive impairment from lipid dysregulation and pro-inflammatory signaling rather than impaired resource allocation *per se*.

1. Introduction

Intensive genetic selection for rapid growth greatly increased meat yield while reducing time to market weight in modern commercial Rock × Cornish (broiler) strains of chicken. However, the improvement of feed efficiency was associated with a tendency to overeat and develop obesity-related morbidities (Griffin and Goddard, 1994; Richards and Proszkowiec-Weglarz, 2007). The best

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described obesity-associated defects in adult birds of current broiler strains are infertility in males and poor egg production (Robinson et al., 1993; Griffin and Goddard, 1994). In the broiler industry, restricting feed intake to approximately 60% of *ad libitum* is usual practice as it prevents obesity, reduces mortality and improves egg production in broiler hens (Leeson and Summers, 2000).

Red Feather, RF, and Black Feather, BF, are two major commercial country chicken strains used for meat production in Taiwan. Despite a much slower growth rate as compared to commercial broilers, the two local strains of chickens account for 40% of chicken meat production in Taiwan due to their meat flavor (Chao et al., 2005a). Even though there is no systematic selection program, modern strains of RF and BF chickens show a greatly improved growth rate as compared to RF and BF strains of the early 1980s (Chao et al., 2005b; Chen et al., 2007). Selection for growth traits and meat production in RF and BF chickens has also been associated with reduced reproductive performance as was observed in broilers undergoing growth selection (Chao et al., 2005b; Kuo et al., 2005; Shiue et al., 2006). There are no reports of whether a restricted feeding program can improve reproductive performance of non-broiler meat-type chicken hens; nor have the effects of different levels of feed intake on lipid accumulation, inflammation or bioactive lipid signaling in relation to ovarian function been reported.

Ad libitum feeding in broiler hens induced obesity-associated dyslipidemia similar to humans with metabolic syndrome (Chen et al., 2006) and some ovarian manifestations resembled those of obese women with polycystic ovary syndrome (PCOS); namely enlarged cystic ovarian follicles in association with follicular inflammation and atresia (Nawrocki and Scherer, 2004; Chen et al., 2006; Goodarzi et al., 2011). Based on these observations we have investigated metabolic changes that occur in feed-satiated broiler hens. Feed-satiated broiler hens exhibited *in situ* lipotoxic and proinflammatory changes including ceramide accumulation, increased interleukin-1β (IL-1β) production, immune cell infiltration, and excessive fatty acid-induced granulosa cell apoptosis in the ovarian hierarchical follicles in conjunction with impaired leukocyte functions related to ovulatory processes (Pan et al., 2012; Xie et al., 2012; Liu et al., 2014).

The present study sought first to determine the impact of restriction of feed intake on the reproductive performance and livability of BF and RF hens. A second aim was to determine whether differences in feed intake affect metabolic processes associated with ovarian function in as similar fashion as was observed in broiler hens.

2. Materials and methods

2.1. Animal management

Two hundred commercial Taiwan country breeder hens of the RF and BF strains reared using regular layer pullet diets were purchased from a local breeder farm at age 14 wk. All pullets were fed *ad libitum* with a soy and corn-based breeder pre-lay mash (11.6 MJ/kg metabolizable energy; 15% crude protein) during a 2 wk adaptation period. At age 16 wk, 50 birds of each strain were continued with sufficient feed for *ad libitum* consumption of a regular layer mash (11.6 MJ/kg metabolizable energy; 17% crude protein) and designated AL-hens. The remaining 50 birds were fed the same diet at a rate of 85% that of the average feed intake of AL-flock during the previous day. These restricted hens were designated as R-hens. Feed was provided at 08:30 AM and hens were maintained on a 14L:10D photoschedule (lights on at 05:00 AM). Hens had free access to water throughout the study. Egg production and feed intake were recorded daily. All bird husbandry and tissue collections were conducted in accordance with an approved animal care protocol in the National Chung University, Taiwan.

2.2. Necropsy, ovarian morphology, and tissue collection

At the plateau of peak egg production at 35 wk, and the end of the laying period at 50 wk, 6 hens with average laying clutch longer than 4 d and having laid an egg within 1 d and within 3 d, respectively, were killed for sampling. Unfed hens were anesthetized with isoflurane prior to collection of blood by heart puncture using EDTA treated tubes, and excision of liver, abdominal adipose tissue, leg and breast muscle, ovarian stroma and follicles. Collected tissues were stored at −80 °C until analyzed. Blood plasma was harvested following centrifugation and stored at −80 °C until analyzed. At the end of the laying period (50 wk) blood was collected from 6 additional hens for plasma analyses. Ovarian morphology was characterized as described previously (Chen et al., 2006) and included enumeration of atretic follicles, incidence of internal ovulation, ovarian involution, and the presence of solid and tumor-like follicles (Bradaric et al., 2013; Johnson and Giles, 2013).

2.3. Collection of follicle granulosa cells

At 35 wk, harvested hierarchical follicles (F1–F5) were quickly soaked in ice-cold Hank's balanced salt solution. Granulosa layers were isolated according to the method of Gilbert et al. (1977). Individual F1, F2 and F3 follicles provided sufficient granulosa cells for analyses to be made for each follicle, however, granulosa cells from F4 and F5 follicles required pooling to provide sufficient material for analysis. For each follicle (or pool), granulosa layers were apportioned into three aliquots (50%, 25% and 25%). The largest portion was rinsed twice with PBS buffer and then dissolved in 2 mL of fetal bovine serum solution containing 10% dimethyl sulfoxide and stored at −80 °C prior to fatty acid and ceramide analysis. A 25% portion was minced and stored in RNAlater (Ambion, Carlsbad, CA, USA) at −80 °C for qRT-PCR analysis. The final 25% portion was used immediately following dispersion by 30 min incubation at 37 °C in M199-Hepes medium containing 200 unit/mL of type-2 collagenase and 0.3 mg/mL of trypsin inhibitor (Sigma, St. Louis, MO, USA) to assess cellular apoptosis. Note that 200 hens were thought to be sufficient to that required numbers of hens meeting the stringent sampling requirements would be available at 35 wk and 50 wk. However, ovarian regression precluded collection of comparable hierarchical follicles between R-hens and AL-hens at 50 wk. For this