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Differential expression of feeding-related hypothalamic neuropeptides in the first generation of quails divergently selected for low or high feed efficiency

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ABSTRACT

Livestock and poultry sectors are facing a combination of challenges, including a substantial increase in global demand for high quality animal protein, general droughts and steady rise in animal feed cost. Thus feed efficiency (FE), which defines the animal's ability to convert feed into body weight, is a vital economic and agricultural trait. Genetic selection for FE has been largely used in chickens and has been applied without knowledge of the underlying molecular mechanisms. Although it has made tremendous progress (breast yield, growth rate, egg production), there have been a number of undesirable changes such as metabolic disorders.

In the present study we divergently selected male and female quail for high and low FE and we aimed to characterize the molecular basis of these differences at the central level, with the long-term goal of maximizing FE and avoiding the unfavorable consequences.

The FE phenotype in first generation quails seemed to be achieved by reduced feed intake in female and increased body weight gain in males. At the molecular level, we found that the expression of feeding-related hypothalamic genes is gender- and line-dependent. Indeed, the expression of NPY, POMC, CART, CRH, melanocortin system (MC1R, MC2R, MC4R, MC5R), ORX, mTOR and ACCα was significantly decreased, however ORXR1/2, AMPKα1, S6K1 and STAT1, 5 and 6 were increased in high compared to low FE males ($P < 0.05$). These genes did not differ between the two female lines. ADPN gene expression was higher and its receptor Adip-R1 was lower in LFE compared to HFE females ($P < 0.05$). In male however, although there was no difference in ADPN gene expression between the genotypes, Adip-R1 and Adip-R2 mRNA abundances were higher in the LFE compared to HFE line ($P < 0.05$).

This study identified several key central feeding-related genes that are differentially expressed between low and high FE male and female quails which might explain the differences in feed intake/body weight gain observed between the two lines. Of particular interest, we provided novel insights into central AMPK–mTOR–ACC transcriptional differences between low and high FE quail which may open new research avenues on their roles in the regulation of energy balance and FE in poultry and livestock species.

1. Introduction

Meeting the substantial increase in demand for high quality animal proteins, that being driven by increasing human population growth, severe drought conditions and diversion of grain to ethanol production (Cahaner and Leenstra, 1992; Chen et al., 2011), will have profound implications for livestock production systems over the coming decades. Since feed is a major component of the total cost (up to 70%) of producing a live bird, feed efficiency (FE) which defines the bird's ability to convert feed into body weight is a key economic and agricultural trait in poultry production.

Genetic selection for increased muscle yield and high growth rate has tremendously improved livestock productivity over the past 50 years (Havenstein et al., 2003), however the selection methods have been applied without knowledge of the fundamental molecular mechanism changes that might be induced by the selection. Indeed, associated with these successes (improved livability, increased muscle yield and growth rate) there have been a number of metabolic muscle disorders in modern chickens such as white striping and muscle myopathy (Wilson et al., 1990), ascites (Julian, 1998), lameness (Hester, 1994), and fat deposition (Griffin and Goddard, 1994). Thus, a deep

Abbreviation: ACCα, acetyl-CoA carboxylase alpha; ADPN, adiponectin; Adip-R1/2, adiponectin receptor 1 and 2; AMPK, AMP-activated protein kinase; ARC, arcuate nucleus; BW, body weight; CART, cocaine and amphetamine regulated transcript; CRH, corticotropin releasing hormone; FAS, fatty acid synthase; FE, feed efficiency; MCR, melanocortin receptor; mTOR, mechanistic target of rapamycin; NPY, neuropeptide Y; ORX, orexin; ORXR1/2, orexin receptor 1 and 2; POMC, proopiomelanocortin; S6K1, P70 S6 kinase; STAT, signal transducer and activator of transcription.

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molecular and cellular understanding of traits of breeding interest may help to avoid the above mentioned unfavorable consequences.

Selection for FE has been largely used in broiler and layer chickens compared to other avian species and has been, for a long time, achieved by estimating the genetic parameters for feed conversion ratio [FCR; feed intake (FI)/weight gain (WG)] and its component traits (Leenstra and Pit, 1988; Gaya et al., 2006; Koerhuis and Hill, 1996). An additional widely used measure of FE in chickens is residual feed consumption or intake (RFC or RFI) which is defined as the difference between the actual and the predicted FI based on the requirements for BW maintenance and growth for broilers (Aggrey et al., 2010) and requirements for BW, growth, and egg production for layer chickens (Bordas et al., 1992).

In the current study Japanese quail (*Coturnix coturnix japonica*) were divergently selected for high and low FE. Japanese quail offer a strong model for chickens and turkeys, while also having favorable characteristics for selection studies (i.e. shortened generation intervals and lowered feed and housing costs). Marks (1996) used coturnix to evaluate the effects of nutritional plane on body weight selection response. Direct selection for feed efficiency is difficult to accomplish with coturnix since they are prone to feed spillage. Therefore, much of the available data regarding feed efficiency is as a trait correlated to body weight selection.

FI is tightly regulated by the hypothalamic satiety and hunger centers (Kuenzel et al., 1999; Sawchenko, 1998) that contain two separate populations of neuronal cell types. One synthesizes orexigenic neuropeptide Y (NPY) and agouti-related peptide (AgRP) (Kuenzel et al., 1987; Phillips-Singh et al., 2003), while the other produces anorexigenic pro-opiomelanocortin (POMC) and cocaine and amphetamine regulated transcript (CART) (Gerets et al., 2000; Tachibana et al., 2003). These neuropeptides interact in a complex way with each other and with the central melanocortin (MCR) system, melanin concentrating hormone (MCH), corticotropin releasing hormone (CRH), orexin, adiponectin, leptin, and ghrelin, to mention a few, to regulate feeding behavior in mammals (for review see (Schwartz et al., 2000)). To better understand the molecular basis of differences between high and low FE male and female quails, we investigate in the present study the expression profile of central feeding-related hypothalamic neuropeptides.

2. Materials and methods

2.1. Animals and experimental design

The present study was conducted in accordance with the recommendations in the guide for the care and use of laboratory animals of the National Institutes of Health and approved by the University of Arkansas Animal Care and Use Committee under protocol # 15039.

The genetic base for the high and low FE quail lines evaluated in this study was derived from a reciprocal cross between the HW quail developed at The Ohio State University (Bacon et al., 1982) and P-line as described by Lepore and Marks (1968). Both quail populations were initiated from a different genetic base and had been independently selected for high 4 wk body for over 50 and 100 generations respectively. Reciprocal crosses between the HW and P-line were conducted to form the AR Heavy currently maintained at the University of Arkansas. The AR Heavy line was maintained and randomly mated for 5 generations in a closed population with 90 breeding pairs. In generation 6 the AR Heavy progeny were subjected to FE testing. Two hatches (n = 555) were placed in FCR cages and tested between 2 and 4 wks of age. High feed efficiency (HFE) and low feed efficiency (LFE) lines were established based on results from phenotypic testing. Once HFE and LFE lines were developed one additional generation of divergent selection was conducted. F_1 progeny (10 females, 3 males/line) with the highest and lowest FE values were selected as the parents of progeny tested in this study.

2.2. Test bird performance

Hatched chicks (n = 150) were reared in one large litter floor pen under thermoneutral conditions with a lighting schedule of 23L:1D. On d14 individual bodyweight (BW) was recorded and chicks were placed in individual cages so feed consumption could be monitored. BW measurements were again taken on d28 when chicks were removed from individual caging. Gain was calculated as (d28 BW – d14 BW) and FE was determined as gain/feed consumption. Males (n = 8) and females (n = 8) with the highest and lowest FE values were selected for brain sampling in this study.

2.3. RNA isolation, reverse transcription and real-time quantitative PCR

Total RNA was extracted from quail brain by TRIzol reagent (Life Technologies, Grand Island, NY) according to the manufacturer's recommendations, DNAse treated and reverse transcribed (Quanta Biosciences, Gaithersburg, MD). RNA integrity and quality were assessed using 1% agarose gel electrophoresis and RNA concentrations and purity were determined for each sample by take 3 μ volume plate using Synergy HT multi-mode microplate reader (BioTek, Winooski, VT). The RT products (cDNAs) were amplified by real-time quantitative PCR (Applied Biosystems 7500 Real-Time PCR system) with Power SYBR green Master Mix (Life Technologies, Grand Island, NY). Oligonucleotide primers used for target chicken hypothalamic genes are summarized in Table 1. The qPCR cycling conditions were 50 °C for 2 min, 95 °C for 10 min followed by 40 cycles of a two-step amplification program (95 °C for 15 s and 58 °C for 1 min). At the end of the amplification, melting curve analysis was applied using the dissociation protocol from the Sequence Detection system to exclude contamination with unspecific PCR products. The PCR products were also confirmed by agarose gel and showed only one specific band of the predicted size. For negative controls, no RT products were used as templates in the qPCR and verified by the absence of gel-detected bands. Relative expressions of target genes were determined by the $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak, 2008).

2.4. Western blot analysis

Brain tissues were homogenized and protein concentrations were determined using Synergy HT multi-mode microplate reader (BioTek, Winooski, VT) as we previously described (Dridi et al., 2012). Proteins (70 μg) were run on 4–12% Novex Bis–Tris gels (Life Technologies, Grand Island, NY). The transferred membranes were blocked for 1 h at room temperature, and incubated with primary antibodies (diluted 1:500–1:1000) at 4 °C overnight. The polyclonal antibodies used were as follows: rabbit anti-human NPY, rabbit anti-human POMC, rabbit anti-mouse ORX, anti-phospho mTOR^{Ser2448}, anti-phospho ACCα^{Ser79}, anti-phospho AMPKα^{Thr172}, anti-mTOR, anti-ACCα, and anti-AMPK. Protein loading was assessed by immunobloting using rabbit anti-GAPDH. Prestained molecular weight marker (precision plus protein Dual color) was used as standard (BioRad, Hercules, CA). All the primary antibodies were purchased from Cell Signaling Technology (Danvers, MA) except for anti-NPY, anti-POMC, and anti-GAPDH from Santa Cruz Biotechnology (Dallas, TX), and anti-ORX from Alpha Diagnostic Int. (San Antonio, TX). The secondary antibodies were used (1:5000) for 1 h at room temperature. The signal was visualized by enhanced chemiluminescence (ECL plus) (GE Healthcare Bio-Sciences, Buckinghamshire, UK) and captured by FluorChem M MultiFluor System (Proteinsimple, Santa Clara, CA). Image acquisition and analysis were performed by AlphaView software (Version 3.4.0, 1993–2011, Proteinsimple, Santa Clara, CA).

2.5. Statistical analysis

Data were analyzed by two-factor ANOVA with gender (male vs. female) and genotype (HFE vs. LFE) as classifiable variables. When

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