

Mathematical modeling of AMPK/mTOR signaling system uncovers the causes in differences of hypothalamic neuropeptide expression regulating feeding behavior in slow- and fast-growing chickens

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Motivation and Aim: Breeding of chickens for high growth rates (HGR) and high body weight has led to changes in the feeding behavior (FB) of meat chicken lines that are unable to adequately regulate feed intake commensurate with their energy needs. This is accompanied by the accumulation of adipose tissue, undesirable body composition, as well as a number of health problems [1]. Thus, for the commercial development of chicken meat lines, a detailed study of the mechanisms underlying the regulation of FB and energy balance of poultry is necessary. The regulation of FB in chickens, like mammals, is mainly carried out by neural networks in the hypothalamus. An important role in the regulation of FB is assigned to specific neuropeptides expressed in the nuclei of the hypothalamus. Depending on their effect on appetite, hypothalamic neuropeptides (HN) have been divided into two groups: orexigenic (OHN) and anorexigenic (AHN). The group of OHNs that increase appetite and create a state of hunger includes neuropeptide Y (NPY) and agouti-related protein (AgRP). AHNs, which include alpha-melanocyte-stimulating hormone (α -MSH), on the contrary, suppress appetite and create a state of satiety. A key role in the regulation of FB is played by the signaling of the hormones insulin and leptin through their influence on the expression of OHNs and AHNs [2]. Hormone signaling through hypothalamic neural networks is closely linked to the adenosine monophosphate-activated protein kinase/mammalian target of rapamycin (AMPK/mTOR) signaling pathway [3]. It has been shown that the expression levels of HNs that mediate the regulation of FB differ greatly between chickens with HGR and slow growth rates (SGR). In meat breeds of chicken, in contrast to SGR chickens, a decrease in the expression level of the proopiomelanocortin (*POMC*) gene, encoding the AHN α -MSH, is observed with age, which may be one of the reasons for excessive feed consumption in broilers [4, 5]. This observation may be based on a difference in signaling of the transcription factor (TF) FOXO1, which is a transcriptional activator of the *POMC* gene. The multiple interactions between AMPK/mTOR and hormone-mediated

signaling pathways, with subsequent regulation of HNs expression, result in complex dynamic behavior of this signaling-metabolic system that is not intuitively predictable. Thus, the application of a mathematical modeling approach, including the development of detailed mechanistic and modular models, is critical to further study the molecular mechanisms and their influence on FB and energy balance in chickens.

Methods and Algorithms: Quantitative metabolomic analysis of chicken tissues was carried out using the NMR method. Metabolomic extracts of chicken heart, liver, kidney, breast and leg muscle were prepared as described in [6, 7], and then NMR spectra of the extracts were obtained using an NMR spectrometer AVANCE III HD 700 MHz (Bruker BioSpin, Germany). For each tissue type, the metabolomic analysis was performed for 12 samples taken from SGR chickens and 12 samples from FGR. Quantification of NMR data was performed by integrating the NMR signals relative to an internal standard. Mathematical models describing the mechanisms of FB regulation in chicken hypothalamic cells with SGR and HGR were reconstructed in the open access BioUML software package [8]. The structure of the developed model is based on the Sonntag model [9], which considers insulin and amino acid signaling as regulators of the AMPK/mTOR signaling system. The signaling pathway of leptin is implemented based on literature data [10] and the mechanisms described in the Yamada model [11]. The structure of the cytosolic and mitochondrial modules, as well as the transport modules, which describe metabolic processes including glycolysis, the tricarboxylic acid cycle, oxidation and biosynthesis of fatty acids, was taken from the model [12], which considers metabolic processes in human skeletal muscles. To expand mathematical models, a cell nucleus module was created, which describes the processes of genetic regulation of the expression of *NPY*, *AgRP* and *POMC* genes encoding HNs.

Results: We determined the concentrations (in units of nmol per gram of a tissue) of 52 metabolites in the chicken breast muscle, 57 metabolites in the leg muscle, 66 metabolites in the heart, 75 metabolites in the kidney, and 74 metabolites in the liver. However, chemometric analysis did not reveal metabolites with statistically significant differences between SGR and FGR chickens. It is possible that the metabolomic differences are too small compared to the variation within each group, and a significantly larger sample size must be used to detect these differences. A composite mathematical model was built, consisting of 6 modules: a cytosol module, which describes the AMPK/mTOR signaling pathway; a nuclear module containing regulation of the expression of HNs; a cytosol and mitochondria modules describing central metabolic processes considering changes in the concentration of metabolites measured by the NMR method, and three transport modules. As part of the modeling of free-feeding conditions for chickens, based on experimental data, approximating functions were proposed that describe changes in leptin concentration in SGR and HGR chickens depending on the age [13]. Mathematical models simulating free-feeding conditions have been fitted to hypothalamic *NPY* and *POMC* mRNA levels in SGR and HGR chickens [14, 15]. The structural differences in the regulation of the expression of these genes were based on the experimentally identified difference between two breeds of chickens: in FGR chickens, the TF FOXO1 was not phosphorylated in the insulin signaling pathway [4, 5]. This mechanism of transcriptional regulation made it possible to obtain differences in the dynamics of expression of the *POMC* and *NPY* genes in accordance with experimental data.

Conclusion: Mathematical models were constructed to describe the regulation of feeding behavior in the cells of the hypothalamus in chickens with a SGR and HGR. The basis

for the structural difference between the models was the observed difference in the signaling of the TF FOXO1, which regulates the expression of HNs. Modeling of the dynamics of expression of the concentrations of the HNs NPY and POMC was carried out under conditions of free feeding of chickens, proposed on the basis of the functions of changes in leptin concentrations in chickens with a SGR and HGR. Structural differences in the FOXO1 signaling pathway mediated differences in the dynamics of POMC and NPY concentrations. Broilers showed an increase in POMC concentrations with age, while chickens with a SGR showed a decrease. This mechanism may explain the difference in FB of meat chicken breeds.

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