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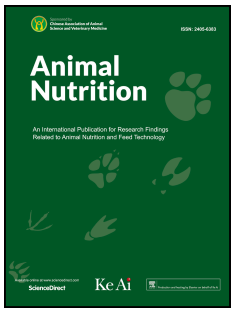

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A bird's-eye overview of molecular mechanisms regulating feed intake in chickens—with mammalian comparisons

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ABSTRACT

In recent decades, a lot of research has been conducted to explore poultry feeding behavior. However, up to now, the processes behind poultry feeding behavior remain poorly understood. The review generalizes modern expertise about the hormonal regulation of feeding behavior in chickens, focusing on signaling pathways mediated by insulin, leptin, and ghrelin and regulatory pathways with a cross-reference to mammals. This overview also summarizes state-of-the-art research devoted to hypothalamic neuropeptides that control feed intake and are prime candidates for predictors of feeding efficiency. Comparative analysis of the signaling pathways that mediate the feed intake regulation allowed us to conclude that there are major differences in the processes by which hormones influence specific neuropeptides and their contrasting roles in feed intake control between two vertebrate clades.

Keywords: Chicken; Feed intake; Hypothalamus; Neuropeptide; Signaling pathway; Hormone

1. Introduction

Molecular mechanisms regulating feed intake during vertebrate ontogenesis are essential for maintaining growth and meat production in livestock, including poultry (Everaert et al., 2019, Richards and Proszkowiec-Weglarz, 2007). Therefore, disentangling these mechanisms orchestrating feeding behavior and energy expenditure is important for commercial breeding and meat industry.

Eating behavior is controlled by central and peripheral regulation, which is coordinated by the nervous and digestive systems. Appetite regulation is provided through the perception of peripheral signals from the external environment and internal physiological signals that convey information about energy and nutritional status (Honda, 2021). The integration of hormonal and nutritional metabolic inputs that control feeding behavior and energy homeostasis is carried out by neural networks in the hypothalamus, “the center of satiety and hunger control”.

Several neuropeptides are expressed in hypothalamic neurons when stimulated by hormones such as ghrelin, insulin, and leptin, providing long-term regulation of eating behavior. Hormone signaling through the hypothalamic neuronal networks is closely related to the adenosine-monophosphate-activated protein kinase/mammalian target of rapamycin (AMPK/mTOR) signaling pathway, which serves as the main sensor of cellular energy. To date, several studies have tackled individual components of signaling pathways that mediate the formation of feed intake in poultry and mammals. However, the understanding of molecular mechanisms regulating feed intake in chickens remains very limited.

It is worth noting that the molecular mechanisms and factors that regulate feed intake in birds

are not comprehensively investigated compared to mammals. In this regard, we overview state-of-the-art knowledge and data on this topic by combining essential differences between two classes of vertebrates and highlighting blank spots in the regulatory mechanisms for chickens.

2. Hypothalamus as a central regulator of feed intake

In birds as well as mammals, the hypothalamus is crucial for controlling feeding behavior by integrating all peripheral and central signals and generating satiety or hunger states. The central nervous system (CNS) receives information about the nutritional and metabolic state via a variety of peripheral signals, including peptide hormones. These signals influence a number of hypothalamic neuropeptides and complex neural circuits in the hypothalamus, which set off the appropriate responses related to feed intake (Kuenzel et al., 1999). The regulation of chicken feed intake and energy homeostasis appears to be comparable to that of mammals, which is implemented by means of neuropeptides produced in the hypothalamic nuclei (Boswell, 2005; Richards and Proszkowiec-Weglarz, 2007). Both mammals and birds are assumed to have satiety areas in the ventromedial and lateral hypothalamus (Kuenzel et al., 1999). In addition to these hypothalamic structures, the paraventricular nucleus (PVN) and the infundibular nucleus (IN), the avian equivalent of the mammalian arcuate nucleus (ARC), are also involved in the control of feed intake (Tachibana and Tsutsui, 2016). Many common neuropeptides between mammals and chickens have been shown to be involved in the control of feed intake (Denbow and Cline, 2015). Two types of hypothalamic neuropeptides that regulate feeding behavior can be identified: some suppress eating behavior and are called anorexigenic neuropeptides, whereas others stimulate eating behavior and are called orexigenic neuropeptides.

Two types of neuron populations in the IN of the hypothalamus are important conduits

through which peripheral signals that affect appetite are integrated (Boswell, 2005; Wynne et al., 2005). One of them expresses the pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) mRNAs, and the other one the neuropeptide Y (NPY) and agouti-related protein (AGRP) mRNAs (Boswell, 2005). Hypothalamic neuropeptides such as adrenocorticotropic hormone (ACTH; also, adrenocorticotropin, or corticotropin) and α-melanocyte-stimulating hormone (α-MSH) derived from the POMC precursor and CART are expected to be anorexigenic in the chickens. NPY and AgRP exert an anabolic effect and are representative candidates for orexigenic neuropeptides (Tachibana, 2016). In addition to orexigenic neuropeptides, these neurons also produce the inhibitory neurotransmitter γ-aminobutyric acid (GABA), which can act in a local circuit to reduce the activity of POMC neurons (Rau and Hentges, 2019). The expression of the neuropeptide genes AGRP, NPY, POMC, and CART was determined in the IN of several species of birds, including chickens, with the expression of AGRP and NPY colocalised in individual IN neurons (Boswell et al., 2002; Boswell and Dunn, 2017; Gerets et al., 2000; Wang et al., 2001; Yuan et al., 2009).

3. Functions of hypothalamic neuropeptides in the feed intake

In mammals and several bird species, including chickens, it is well known that the neuropeptide gene expression in neurons of the arcuate nucleus is affected by nutritional status and changes in energy levels (Boswell and Dunn, 2017). In recent decades, numerous studies have been conducted to explore the function of orexigenic AgRP, NPY, and anorexigenic α-MSH neuropeptides that regulate feeding behavior in chickens (Tables 1 and 2).

The POMC and AGRP genes encoding neuropeptides, along with melanocortin receptors (MC-R), constitute the central melanocortin system (Boswell and Dunn, 2015). MC-R neurons

project from the mammalian arcuate nucleus to the PVN, containing a high density of MC-R. Activation of these receptors causes a decrease in food consumption and an increase in energy use (Gali Ramamoorthy et al., 2015).

Chicken melanocortin signaling in the hypothalamus is mediated by specific subtypes of MC-R, i.e., melanocortin receptors 3 (MC3R) and 4 (MC4R), by binding to such agonists as AgRP and α-MSH/ACTH (Boswell and Dunn, 2015). Neuropeptides α-MSH and ACTH act as activators of chicken melanocortin receptors (Zhang et al., 2017). As expected, the central injection of α-MSH in broiler neonatal chicks followed by administration of an MC4R antagonist led to the reduction of the α-MSH anorexigenic effect, suggesting that its effect is MC4R-mediated (Saneyasu et al., 2011a). Indeed, α-MSH mediated anorexigenic effect in chickens, suppressing feed intake in both broilers and layers after intracerebroventricular (ICV) injection (Cline and Smith, 2007, Honda et al., 2007, 2012; Kawakami et al., 2000; Saneyasu et al., 2011a). In contrast, β-MSH causes an anorexigenic effect in layers (Honda et al., 2012), but not in broiler chickens (Honda et al., 2012, Saneyasu et al., 2011a).

By contrast to α-MSH and ACTH, AgRP can act as an inverse agonist for MC-Rs. Furthermore, AgRP can also antagonise α-MSH/ACTH action on these receptors (Zhang et al., 2017). Indeed, central injection of AgRP with α-MSH attenuated the anorexigenic effect of α-MSH in both neonatal broilers and layer chicks (Tachibana et al., 2001, Kawakami et al., 2000). Under ad libitum feeding settings, AgRP injection increased food consumption in neonatal layer chicks but not in broilers, indicating that the orexigenic impact of endogenous AgRP varies between the two breeds, at least at the neonatal stage (Tachibana et al., 2001).

In both chickens and mammals, NPY is regarded as a powerful stimulant of feeding behavior

(Greene et al., 2022). Indeed, central injections of NPY resulted in stimulation of feed intake in broilers (Ando et al., 2001; Saneyasu et al., 2011b) and slow-growing chicks (Tachibana et al., 2001, Saneyasu et al., 2011b). After four d of food deprivation, NPY mRNA and peptide levels increased markedly in the hypothalamic IN nuclei of layers, suggesting its involvement in the regulation of feed intake (Kameda et al., 2001).

Fasting for 24 and 48 h upregulated the hypothalamic NPY and AgRP gene expression and downregulated POMC in yellow-feathered broiler chicks (Fang et al., 2014). In young Arbor Acres broilers fasted for 48 h, the mRNA expression levels of orexigenic neuropeptides were increased too, but the gene expression of POMC was not affected by the starvation (Song et al., 2012). However, newly hatched broiler chicks after the same period of fasting showed a significant increase in POMC mRNA (Higgins et al., 2010). The discrepancies between the effects of fasting on POMC gene expression is supposed to be due to different breeds and ages of broiler chickens.

Differences in the expression of hypothalamic neuropeptides should also be caused by the period of fasting. Because in Arbor Acres broiler chicks of the same age under 24-hour feed restriction conditions, NPY mRNA levels in the hypothalamus were similar to those in ad libitum-fed chicks. At the same time, starvation led to activation and inhibition of hypothalamic AgRP and POMC gene expression respectively. Refeeding following 24 h of fasting increased mRNA levels of POMC, but decreased mRNA levels of AgRP (Liu and Zhu, 2012). One can assume the increase in appetite during fasting was due to the suppression of the anorexigenic POMC gene expression, and the activation of the orexigenic AgRP, but not the NPY. This is confirmed by the fact that after refeeding the POMC mRNA levels were increased and the AgRP mRNA levels

were decreased, and serve as an indicator of satiety.

When fasting for a shorter period of time, 12 h, gene expression levels of NPY in both Ross 308 broiler and layer chicks were significantly elevated and returned to control levels after 12 h of refeeding. In contrast, upregulation of AgRP after starvation was observed only in broilers, and these changes were not reversed by refeeding. Simultaneously, starvation did not influence the mRNA levels of hypothalamic POMC in either layer or broiler chicks (Kewan et al., 2021). It is likely that 12 h of refeeding is not enough to suppress feed intake in broilers, and appetite control is probably achieved through upregulation of AgRP, but not NPY.

Prolonged feed restriction of Ross 308 broilers for 6 wk showed increased levels of AgRP mRNA, which returned to control levels after unlimited access to food for 2 d. At the same time, observations were found for NPY, although changes in expression level were not as significant (Dunn et al., 2013). Feed restriction did not change the expression of anorexigenic POMC gene, which was also observed during 12-h fasting in another experiment with Ross 308 (Dunn et al., 2013, Kewan et al., 2021).

When chickens were restricted to feed for 7 d, a significant reduction was identified in POMC hypothalamic expression in both Cobb broilers and layer chicks. However, the suppression of POMC gene expression was more pronounced in layers than in broilers (Hen et al., 2006).

In mammals, α-MSH induces a release of corticotropin-releasing factor (CRF) in a hypothalamic PVN, an area that controls both the hypothalamic-pituitary-adrenal axis (HPA) and feeding behavior (Lu et al., 2003). An anorectic action of CRF is observed in mammals, as well as in chickens, for which it was found to suppress feed intake after central administration in both broilers and layer hens (Denbow et al., 1999). In order to ascertain how α-MSH and CRF neurons

interact in chickens, there was a study on the effect of ICV α-MSH injection on corticosterone (CORT) secretion, which is the main stress hormone in birds and is produced when HPA is activated (Tachibana et al., 2007). In particular, it was revealed that in layer chickens, CORT release is induced by central administration of α-MSH (Tachibana et al., 2007). An increase in CORT levels was also observed in broiler chickens after ICV injection of β-MSH (Smith et al., 2007). Moreover, the significantly increased level of hypothalamic CRF mRNA was detected in neonatal broilers after central administration of β-MSH, proposing that CRF participates in the β-MSH anorexigenic pathway (Saneyasu et al., 2013).

4. Feed intake in the hypothalamus is controlled by the AMPK/mTOR signaling pathway

In chickens, there is strong evidence for an association between the control of central melanocortin signaling by hypothalamic energy perception and neuropeptide gene expression in the hypothalamus. At the cellular and organismal levels, AMPK is a central energy sensor essential for maintaining energy homeostasis. AMPK controls energy balance by integrating a diverse set of physiological signals, such as nutrition status and the metabolic effects of hormones. In the hypothalamus, AMPK completes crucial functions in the control of feed intake and maintaining energy balance and body weight (Hardie, 2014). AMPK signaling is activated under low-energy conditions, elevating energy production and reducing energy consumption. AMPK activation restricts energy expenditure by inhibiting anabolic processes and stimulating catabolic processes, in an attempt to restore cellular energy charge.

The mammalian AMPK complex is composed of one α-catalytic subunit and two regulatory β- and γ-subunits. Chicken AMPK (chAMPK) subunits were shown to have considerable

homology to the amino acid sequence of human AMPK (Proszkowiec-Weglarz et al., 2006). This may indicate the similarity of AMPK functioning in chickens and mammals (Fig. 1).

Initial research suggests that AMPK activation occurs due to an increase in the cellular ratio of adenosine monophosphate/adenosine triphosphate (AMP/ATP) and under physiological conditions of negative energy balance, including hunger. Furthermore, AMPK can be activated by direct allosteric binding of AMP. However, there are studies demonstrating that some hormones (e.g., ghrelin) can induce AMPK activity regardless of a change in the AMP/ATP ratio. On the contrary, inhibition of AMPK activity is observed under energy-sufficient conditions (feeding, reduction in AMP/ATP, insulin, and leptin) (Ronnett et al., 2009). In addition, there is evidence that AMPK activation is promoted by phosphorylation of Thr172 in the catalytic domain by the upstream kinases: liver kinase B1 (LKB1) and Ca2+/calmodulin-dependent protein kinase β (CaMKKβ).

Immunocytochemical analysis revealed the localization of phosphorylated AMPK in IN, PVN, and other hypothalamic nuclei in chickens, which are closely related to the regulation of feed intake and energy homeostasis. While AMPK phosphorylation was shown to be influenced by nutritional status. Restriction of broiler chickens feeding affected the decrease in the ratio of phosphorylated AMPK to the total amount in the hypothalamus. On the contrary, repeated feeding contributed to a decrease in the level of phosphorylated AMPK (Proszkowiec-Weglarz et al., 2006).

Using the immunofluorescence method, it was shown that the LKB1 protein, a major AMPK upstream kinase, was expressed in chicken hypothalamic cells (Zhang et al., 2021). The starvation of broiler chickens contributed to an increase in hypothalamic levels of phospho-LKB1 compared

to total LKB1 (Proszkowiec-Weglarz et al., 2006).

Analysis of AMPK gene expression in the brain, including the hypothalamus, of broiler chickens revealed priority expression of the α1, β2 and γ1 subunit isoforms. However, alterations in energy status (starvation and feed intake) did not contribute to a significant change in the transcription of the AMPK subunit genes (Proszkowiec-Weglarz et al., 2006). A study of the effect of dietary energy level on the AMPK signaling pathway in the hypothalamus of broiler chickens showed that a high-energy diet led to suppression of appetite and expression of the *LKB1* and *AMPKα1* genes. On the contrary, a low-energy diet increased AMPKα2 mRNA levels and increased appetite (Hu et al., 2019). Based on the above, it can be concluded that energy availability affects hypothalamic chAMPK, as in mammals. Therefore, the data suggest that the LKB1/AMPK hypothalamic signaling pathway exists, at least in broilers. However, the functionality of the CaMKKβ/AMPK pathway in the chicken hypothalamus requires further study.

A crucial role in the regulation of feeding behavior and maintaining energy balance is assigned to mTOR signaling in the hypothalamus, which responds to changes in nutrient status (Cota et al., 2006). mTOR is a serine-threonine kinase and is a component of two multiprotein complexes, mTORC1 and mTORC2, which have different structures and functions. In the hypothalamus, mTORC1 acts as a sensor of changes in nutrient and energy status in rats: its activity increases with feed intake and decreases with fasting (Cota et al., 2006). The activity of mTORC1 is regulated in response to growth factors, hormones (including leptin, insulin, and ghrelin), and nutrient signaling (glucose and amino acids) (Hu et al., 2016). Amino acids can control the activity of mTORC1 through the Rag proteins (recombination-activating gene), that is, a set of small GTPases (Sancak et al., 2008). Branched-chain amino acids, such as L-leucine

and L-arginine, are considered potential activators of mTORC1 (Jewell et al., 2013). ICV injection of L-leucine into broilers and layer chicks significantly stimulated feed intake, while L-arginine did not significantly affect broiler chicken feed intake (Kehinde et al., 2022; Wang et al., 2012).

Growth factors and insulin activate the phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) signaling pathway (a detailed description is presented below), causing the phosphorylation of serine/threonine kinase, protein kinase B (Akt). Phosphoinositide-dependent protein kinase 1 (PDK-1) is an upstream kinase of Akt, which activates Akt by phosphorylation at Thr308. However, mTORC2 is required for maximal Akt activation, which is achieved through phosphorylation at Ser473 (Dibble and Cantley, 2015). This is accomplished by increasing the activity of mTORC2 due to its phosphorylation by Akt, forming a positive feedback loop with each other (Yang et al., 2015). It was identified that feeding after a fast led to a significant elevation of phosphorylated Akt (Thr308), but not Akt (Ser473) levels in the hypothalamus of layer and broiler chickens (Saneyasu et al., 2018, 2019).

Activated Akt inhibits the tuberous sclerosis complex (TSC1/2), which acts as a GTPase activating protein for the small GTPase Rheb (Ras homologue enriched in brain), through multiple phosphorylation of the TSC-1 subunit. This contributes to mTORC1 activity stimulation by suppressing Rheb (Inoki et al., 2002).

Similar to AMPK, mTORC1 is involved in energy perception (Fig. 1). However, mTORC1 has the opposite effect of AMPK under conditions of high cellular energy levels. Besides that, AMPK stimulation results in mTORC1 inactivation. Low available cellular energy due to glucose restriction inhibits mTORC1 via activation of AMPK. Two AMPK-catalyzed phosphorylation

events counteract the activating effects of Akt on mTORC1: (1) phosphorylation of the TSC-2 subunit in the TSC1/TSC2 complex, which suppresses Rheb-GTP-dependent mTORC1 activation; and (2) AMPK-mediated phosphorylation of the regulatory-associated protein of mTOR (Raptor). The latter is an essential regulatory mTORC1 subunit whose phosphorylation is required for mTORC1 kinase activity inhibition (Xu et al., 2012).

The central administration of Compound C, an inhibitor of AMPK, reduced feed intake in broiler chickens and caused a great decrease in hypothalamic AMPKα phosphorylation and an increase in mTOR phosphorylation. This may indicate that AMPK signaling in the hypothalamus participates in the feed intake control in broiler chickens (Hu et al., 2021). There was also a study in which layer chicks were ICV injected with rapamycin, the mTOR inhibitor, causing inhibition of feed intake (Saneyasu et al., 2018). This suggests that rapamycin blocks hypothalamic mTOR signaling in chickens.

mTORC1 senses alterations in nutrient and hormone levels and regulates translational control of protein synthesis by binding the two downstream targets to Raptor and phosphorylating them. Stimulation of mTORC1 signaling resulted in inactivation of the mRNA translation repressor, the 4E binding protein of eukaryotic initiation factor 4E-binding protein 1 (4E-BP1), and activation of ribosomal protein S6 kinases (S6K) (Hay and Sonenberg, 2004). Upon refeeding after fasting, a significant increase in phosphorylated S6K1 and S6 (downstream target of S6K1) was noted in rats (Cota et al., 2006). In layer chicks fed after a 24-h fast, the level of hypothalamic phosphorylated ribosomal protein S6 increased significantly (Saneyasu et al., 2018). However, refeeding after fasting did not alter hypothalamic S6 phosphorylation in broiler chickens (Saneyasu et al., 2019).

5. Hormonal regulation of feed intake

The hypothalamus integrates information from hormones such as insulin, leptin, and other peptide hormones secreted by the gastrointestinal tract, liver, and adipose tissue.

As a rule, the influence of signaling peptides synthesized in the intestines has a short-term effect on appetite, which in turn does not have a significant role in mediating long-term changes in energy balance and body weight. However, some hormones can contribute to long-term changes in energy balance by activating or inhibiting metabolic pathways (Tables 3 and 4). Ghrelin and leptin have been recognized as key hormones that significantly influence the long-term regulation of energy balance in chickens and mammals. Ghrelin is known as a "hunger hormone" in mammals, because it drives short-term food consumption and manages long-term body weight control (Higgins et al., 2007). Unlike mammals, ghrelin has the opposite effect on feeding behavior in chickens and leads to decreased feed intake (Murugesan et al., 2022). Approximately two decades have passed since the discovery of leptin as a satiety hormone in mammals (Friedman and Halaas, 1998). Later on, the leptin gene in chickens was finally identified and cloned (Seroussi et al., 2016). Leptin is believed to serve as a communication link between peripheral fat reserves and the CNS (Friedman, 2014). Nevertheless, recent findings suggest that this relationship does not hold true in chickens (Friedman-Einat and Seroussi, 2019). In chickens, insulin's function is somewhat conserved compared to mammals and, like leptin, is thought to act as an appetite suppressant peptide. Although significant differences exist in insulin sensitivity and glucose homeostasis, chickens are naturally more glucose intolerant and insulin resistant (Seki et al., 2003). However, it is likely that, depending on the age and breed of chickens with high and low growth rates, different effects are found in the influence of these hormones on

feed intake in chickens.

5.1. Insulin

In mammals, the pancreatic hormone insulin is defined as an adiposity signal that regulates blood glucose levels (Woods and Seeley, 2001). However, there is evidence that most likely insulin does not function as an adiposity signal in birds. Since components of the insulin signaling pathway in chicken adipose tissue were found to be insulin insensitive (Dupont et al., 2012), plasma insulin levels and the abdominal fat mass were unrelated (Honda et al., 2015).

According to numerous studies ICV insulin injection suppressed feed consumption in slow-growing chickens (Honda et al., 2007; Shiraishi et al., 2008, 2009, 2011b). The ICV insulin administration to Chunky broiler chickens did not affect their feed intake (Shiraishi et al., 2011b). However, in Ross 308 broilers central insulin injection decreased feed consumption in a dose-dependent manner (Yousefvand et al., 2018, 2020). This difference in insulin-mediated feed intake may be due to different breeds of broiler chickens. Peripheral insulin treatment also did not affect changes in the feed intake of broiler chicks (Liu et al., 2016).

5.1.1. Insulin-dependent signaling pathways that control eating behavior

In chickens, insulin receptors were located in several structures of the hypothalamus, while in IN, the presence of insulin receptors was found both in anorexigenic POMC/CART neurons and in orexigenic AgRP/NPY neurons (Shiraishi et al., 2011a). The levels of InsR expression in the hypothalamus varied between broilers and layer chickens. Under conditions of free access to feed, the expression of InsR in broilers is considerably lower compared to layers, which was accompanied by increased insulin concentrations in broilers. Moreover, feed restriction substantially downregulated the InsR expression only in layer chicks, which together may indicate

insulin resistance in broiler chicks (Shiraishi et al., 2011b).

Insulin receptor structure is conserved between chickens and mammals. The α subunit of InsR is the insulin-binding subunit, while the β subunit exhibits insulin-stimulated tyrosine-specific autophosphorylation (Simon and Leroith, 1986). In its inactive form, the insulin receptor exists as a dimer (Ottensmeyer et al., 2000).

Insulin binding to the receptor results in autophosphorylation of tyrosine residues among β subunits, which allows binding to the insulin receptor substrate (IRS) protein family. It was determined that the insulin receptor substrate 1 (*IRS-1*) gene is expressed in the brain of chickens and phosphorylated at tyrosine residues in response to insulin, at least in the LMH cell line derived from a Leghorn chicken hepatocellular carcinoma that was previously shown to be insulin sensitive (Taouis et al., 2009).

The phosphorylation at tyrosine residues activates IRS proteins and enables PI3K recruitment to the cell membrane and subsequent activation. Phosphorylated IRS proteins interact with the PI3K regulatory subunit (p85), which contributes to activation of the PI3K catalytic subunit (p110), allowing it to phosphorylate membrane-bound phosphatidylinositol 4,5-bisphosphate (PIP2) to promote phosphatidylinositol (3,4,5)-triphosphate (PIP3) synthesis (Engelman et al., 2006). This mediates membrane translocation of serine/threonine kinases PDK-1 and Akt that are binding to membrane-bound lipid PIP3, which leads to activation of Akt by phosphorylation at Thr308 (Boucher et al., 2014). Akt activation in response to insulin promotes IRS-1 phosphorylation on serine residues, which creates a positive feedback loop for insulin action (Gual et al., 2005).

Central injection of LY294002, a PI3K inhibitor, significantly facilitated feed intake in

starving layer chicks. In a separate study, injection of LY294002 significantly prevented insulin-induced elevation in hypothalamic phosphorylated Akt activity (Thr308), indicating that LY294002 inhibits PI3K in the hypothalamus of layer chickens (Saneyasu et al., 2018). The research mediated by the signaling pathway in White Leghorn layer hens and Ross 308 broilers showed that ICV insulin injection reduced feed intake and significantly increased Akt and S6 phosphorylation in the chicken hypothalamus (Saneyasu et al., 2018, 2019).

In mammals, the hypothalamic mTOR/S6K signaling acts as a negative regulator of PI3K-related signaling. Because S6Ks phosphorylate IRS-1 at several serine residues to promote inhibition of insulin signaling at the IRS-1 level (Blouet et al., 2008). In broiler chickens, it was demonstrated that repeated feeding and central insulin administration led to increased IRS-1 serine residue phosphorylation but did not affect the phosphorylation of tyrosine residues in skeletal muscles. This suggests the possibility of a negative feedback mechanism, which may reduce the activity of IRS-1 by increasing the phosphorylation of serine residues. (Duchêne et al., 2008).

In mammals, leptin binding to the receptor leads to the activation of the JAK2 (Janus tyrosine kinase 2) protein, which exists in complex with the receptor, which leads to the activation of IRS-1 and IRS-2, which in turn are phosphorylated by JAK2. Therefore, IRS-1 is a cross-component of the insulin and leptin signaling pathways, indicating that leptin and insulin regulatory effects on appetite may be achieved through the IRS-1/PI3K interaction (Barrios-Correa et al., 2018).

The FOXO1 (forkhead box protein O1) transcription factor is the downstream target of Akt. In mammals, hypothalamic FOXO1 activation promotes increased feed consumption and body mass, while FOXO1 inhibition has the opposite effect. FOXO1 acts as an activator of the

orexigenic neuropeptides NPY and AgRP transcription, and as an inhibitor of anorexigenic POMC transcription (Kim et al., 2006). This action is opposite to the effects of the leptin-stimulated activated transcription factor STAT3 (signal transducer and transcription 3), which inhibits AgRP and NPY expression and activates POMC (a detailed description is presented below). Additionally, an increase in the level of FOXO1 expression leads to the formation of a complex with activated phosphorylated STAT3 in the nucleus, blocking binding of STAT3 with the POMC promoter, which contributes to the inhibition of POMC expression activation mediated by leptin signaling (Yang et al., 2009).

Insulin suppresses feed intake by activating the PI3K/Akt signal, leading to inhibition of FOXO1 activity. Once fully activated, Akt becomes capable of phosphorylating its targets, including FoxOs (Manning and Cantle, 2007). Activated Akt induces FOXO1 phosphorylation, followed by exclusion from the nucleus and subsequent proteasomal degradation. Therefore, activation of the PI3K/Akt pathway leads to inhibition of FOXO1 activity, which contributes to a decrease in the expression of orexigenic neuropeptides while simultaneously activating STAT3-mediated transcription of POMC by decreasing the antagonistic effect of FOXO1 on STAT3 (Kodani and Nakae, 2020).

The PI3K/Akt-mediated pathway study in broiler chickens indicated that central insulin administration in contrast to mice (Kim et al., 2006) had no impact on phosphorylated FOXO1. A t the same time, insulin did not significantly affect the hypothalamic neuropeptide POMC gene expression (Saneyasu et al., 2019). These may indicate that expression of POMC induced by leptin signaling is not associated with the function of FOXO1 in the hypothalamus, which may be one of the reasons for excessive feed intake in broiler chickens. However, ICV insulin

administration in layer chicks increased the level of FOXO1 phosphorylation and hypothalamic POMC expression (Saneyasu et al., 2018). Indeed, the level of hypothalamic POMC and CART mRNA significantly increased after central insulin injection in layer chicks (Honda et al., 2007; Shiraishi et al., 2008). At the same time, insulin ICV injection could inhibit or not affect NPY gene expression (Shiraishi et al., 2008, Honda et al., 2007). Furthermore, the central injection of insulin did not change AgRP gene expression (Honda et al., 2007; Shiraishi et al., 2008). A summary of the insulin signaling pathway and observed differences between chickens and mammals has been summarized in Fig. 2.

5.2. Leptin

The adiposity hormone leptin performs a crucial function in the regulation of feeding behavior and energy balance in mammals. Mammalian leptin is predominantly secreted by adipose tissue and acts as a transmitter of body fat information (Friedman, 2014). However, leptin expression is not limited to adipose tissue, the stomach and duodenum also produce a significant amount of leptin (Cammisotto and Bendan, 2012).

In birds, including commercial breeds of chickens (broiler and layer chickens), unlike mammals, leptin is not expressed in adipose tissue, and the expression levels of this hormone do not correlate with obesity (Bornelöv et al., 2018; Farkašová et al., 2016; Huang et al., 2014; Resnyk et al., 2015). This suggests that leptin is not a key signal for fat stores (Friedman-Einat and Seroussi, 2019).

In most studies, leptin expression levels in birds, including chickens, were shown to be especially high in the brain, including the cerebellum, hypothalamus, and pituitary gland (Farkašová et al., 2016; Friedman-Einat et al., 2014; Huang et al., 2014; Seroussi et al., 2016). At

406 the same time, the leptin expression patterns in these tissues closely correlated with the expression
407 level of LEPR, indicating a paracrine/autocrine mode of action of this hormone in birds
408 (Friedman-Einat and Seroussi, 2019). The expression of chicken leptin and leptin receptor (LEPR)
409 has also been found in the digestive system (duodenum, caecum, ileum, and pancreas). Leptin is
410 observed in the duodenal mucosa, suggesting that it is involved in short-term appetite regulation
411 (Seroussi et al., 2019).

412 Leptin regulates eating behavior by binding to leptin receptors in hypothalamic neurons, with
413 the ARC nucleus being the main center sensitive to leptin. In mammals, leptin contributes to the
414 activation of POMC/CART neurons and the suppression of the activity of AgRP/NPY neurons
415 through the corresponding signaling transduction pathway (Van Swieten et al., 2014).

416 There are many studies that examine the effect of leptin injection on feed intake in chickens.
417 However, the results are quite inconsistent, demonstrating both inhibition of feeding and no effect
418 on appetite in birds. Central administration of recombinant human leptin promotes lower feed
419 intake in broilers and layer chickens (Denbow et al., 2000). However, in slow-growing White
420 Rock chickens selected for body weight, human recombinant leptin caused a reduction in feed
421 intake only in chickens with low body weight (Kuo et al., 2005). ICT injection of the incomplete
422 synthetic chicken leptin peptide did not affect feed intake in Hubbard x Cobb-500 broiler chicks
423 (Sims et al., 2017). As found in another study, leptin contributed to a significant inhibition of feed
424 intake in Ross 308 broiler chicks (Adeli et al., 2020). There was also a study that examined the
425 effect of intraperitoneal injection (IP) of recombinant chicken leptin in broilers and layers of two
426 age groups. In young and adult layers, IP leptin administration resulted in appetite inhibition,
427 while young broilers had no significant effect on feed intake (Cassy et al., 2004). Although the

differences between the results of different studies remain incompletely identified, it is possible that the breed, age, or source of leptin (human or chicken recombinant leptin) are responsible for the observed distinction.

5.2.1. Leptin-dependent signaling pathways that control eating behavior

To date, the leptin signaling pathway involved in the control of feeding behavior in chickens is poorly understood. However, the experimental data demonstrate the conservative basics of similar signaling pathways in mammals (Fig. 3).

The leptin signaling pathway initiates through the binding of leptin to specific receptors, leptin receptors. This, in turn, promotes activation of several signaling pathways, including JAK2/STAT3 and PI3K/IRS/Akt, which mediate the regulation of feed intake and energy homeostasis. The leptin signaling pathway contributes to inhibition of hypothalamic AMPK through the PI3K/Akt pathway, inducing p70S6K-dependent direct phosphorylation of the AMPK α-subunit at Ser491 t (Dagon et al., 2012). However, ICV leptin administration in broiler chickens activated AMPK, significantly facilitating AMPK phosphorylation at Thr172 of the α-subunit in the hypothalamus (Piekarski et al., 2018). A summary of the leptin signaling pathway and observed differences between chickens and mammals has been summarized in Fig. 3.

Several isoforms of the leptin receptor (chLEPR) exist in chickens. The long isoform of the leptin receptor (chLEPRb) contains JAK2 and signal transducer and activator of transcription 3 (STAT3) binding motifs and three conserved mammalian tyrosine residues (Tyr-986, Tyr-1079, and Tyr-1141) associated with intracellular domain phosphorylation. The short isoform of the chLEPR lacks the STAT3 binding motif and contains only the JAK2 binding motif. Only chLEPRb is able to activate the JAK2/STAT3 pathway. It should be noted that in chickens, there

is a lack of expression of the short forms of the chLEPR in the brain, while the long isoform demonstrates a high level of expression, including in the hypothalamus (Liu et al., 2007). Central leptin injection promoted the expression of chLEPRb in the hypothalamus (Piekarski et al., 2018).

In mammals, binding of leptin to the long isoform of the leptin receptor (LEPRb) leads to dimerisation of the receptor subunits. As a result, JAK2 associated with the intracellular domains of receptors is activated through autophosphorylation due to their proximity to each other (Mengie Ayele et al., 2022). Activated JAK2 stimulates the phosphorylation of three tyrosine residues of the LEPRb intracellular domain (Tyr 985, Tyr1077, and Tyr1138) to create binding sites for proteins. It further enables STAT3 to bind to the receptor at phosphorylated Tyr1138. Then STAT3 is phosphorylated by JAK2 (Liu et al., 2021). ChLEPR was revealed to activate the JAK2/STAT3 signaling pathway in vitro. Stimulation with leptin resulted in STAT3 phosphorylation via chLEPR and JAK2 (Adachi et al., 2008). This proves that vertebrates share a similar leptin signaling pathway.

The phosphorylation of STAT3 promotes its dimerisation and translocation to the nucleus, where it acts as a transcriptional regulator of genes, including suppressor of cytokine signaling 3 (SOCS3) and neuropeptides (POMC, AGRP, and NPY) (Banks et al., 2000; Kwon et al., 2016). In chickens, leptin induced STAT3 phosphorylation and its subsequent translocation to the nucleus in COS-7 cells expressing chLEPR (Adachi et al., 2012). SOCS3 acts as a feedback inhibitor of the JAK2/STAT3 signaling by interacting with LEPR or JAK2, thereby blocking STAT3 activation (Bjørbæk et al., 2000). Like in mammals, SOCS3 in chickens was demonstrated to be a feedback inhibitor of leptin signaling. This mechanism, however, might be a little different from that found in mammals. Chicken SOCS3 inhibits leptin signaling by binding directly to

JAK2, then blocking phosphorylation and subsequent activation of STAT3. SOCS3 may not interact with phospho-Tyr986 in the intracellular domain of chLEPR for leptin signaling inhibition (Adachi et al., 2013).

The transcription factor STAT3 binds to the promoters of genes that encode anorexigenic neuropeptides (POMC) and anorexigenic neuropeptides (AgRP and NPY). STAT3 acts as an activator of POMC expression and promotes the down-regulation of AGRP and NPY expression, thus reducing feed intake and inducing energy expenditure in mammals (Liu et al., 2021).

However, the mechanism of neuropeptide-mediated action of leptin in chickens may differ from that established in mammals. ICT administration of recombinant chicken leptin decreased the hypothalamic expression of the orexigenic neuropeptide NPY in broilers. However, no changes in AGRP and POMC expression were observed (Dridi et al., 2005). This may suggest that, at least in broiler chickens, leptin preferentially acts through orexigenic neuropeptides (NPY, but not AgRP) as opposed to anorexigenic pathways (POMC). In particular, after immunization against chLEPR, the hypothalamic expression of AGRP and NPY was upregulated, whereas the expression of POMC was significantly downregulated (Lei et al., 2015).

5.3. Ghrelin

In mammals one of the crucial peptides involved in controlling appetite and energy homeostasis is ghrelin. Ghrelin also has a stimulating effect on growth hormone (GH) secretion (Kojima et al., 1999). In young chicks, the chicken ghrelin injection also transiently increased plasma GH concentrations (Kaiya et al., 2002).

In mammals, ghrelin is an orexigenic hormone released predominantly by the gastric mucosa, although it is widely expressed in many different tissues, including the central nervous system,

494 the gastrointestinal tract, and the pituitary gland (Devesa, 2021). In the case of layers, chicks had
495 ghrelin mRNA at the highest levels in the proventriculus, which was comparable to the gastric
496 fundus, but not in the gizzard, whose function is to mechanically process food (Kaiya et al., 2002).
497 This was also shown in another study in which the highest expression was in the proventriculus
498 and then in the pancreas, brain, and intestines in broiler chickens (Richards et al., 2006). These
499 results indicate that the major site of ghrelin synthesis in laying chickens and broiler chickens is
500 the same, regardless of their lineage. Ghrelin immunopositive cells were found in the mucosal
501 layer of the proventriculus, gastrointestinal tract, and chicken hypothalamus (Ahmed and Harvey,
502 2002; Neglia et al., 2004; Wada et al., 2003).

503 The role of ghrelin in relation to feeding behavior and energy balance in chickens differs
504 from that in mammals. In mammals, ghrelin acts as an appetite stimulating hormone both after
505 central and peripheral ghrelin injection, but in chickens, central ghrelin administration, in contrast,
506 suppresses feed intake (Furuse et al., 2001; Saito et al., 2002a, 2005; Taati et al., 2010). In
507 mammals, ghrelin modulates feeding behavior through the growth hormone secretagogue
508 receptor (GHS-R) in hypothalamic neurons, including the ARC nucleus, a main center for
509 maintaining energy homeostasis. Ghrelin stimulates orexigenic AgRP/NPY-associated neurons
510 and inhibits anorexic POMC neurons in the hypothalamus, increasing feed intake and body mass
511 (Kageyama et al., 2010).

512 Several studies revealed that central ghrelin injection suppressed feed consumption in both
513 broilers and layer chickens (Furuse et al., 2001; Saito et al., 2002a, 2005; Taati et al., 2010).
514 Surprisingly, the effect of peripheral ghrelin injections on feed intake shows conflicting results
515 between chicken strains. Intravenous injections of chicken ghrelin did not influence feed intake

among layer chickens (Kaiya et al., 2007). In contrast, peripheral injections of ghrelin into newly hatched and young broiler chickens suppressed feed intake (Buyse et al., 2009; Geelissen et al., 2006, Ocłoń and Pietras, 2011).

Further evidence points to a fundamental difference between the peripheral action of ghrelin in chickens and mammals. Peripheral injection of ghrelin into broiler chickens resulted in increased expression of the key lipogenic enzyme fatty acid synthase (FAS) and its associated transcription factors, sterol regulatory element binding protein-1 (SREBP-1) and peroxisome proliferator-activated receptor-γ (PPARγ) in the diencephalon. These findings imply that the anorectic action of ghrelin is mediated by central fatty acid metabolism. On the other hand, the decreased expression levels of FAS and both transcription factors were significantly observed in the liver. This result suggests that ghrelin has a peripheral antilipogenic effect in chickens (Buyse et al., 2009). Intravenous ghrelin injection was found to be accompanied by a reduction in respiratory quotient in broiler chicks, while heat production was not changed, suggesting a decrease in de novo lipogenic activity (Geelissen et al., 2006). The impact of ghrelin in animals, which encourages an increase in respiratory quotient and the deposition of fat, is contrary to this antilipogenic function (Kaiya et al., 2013).

5.3.1. Ghrelin-dependent signaling pathways that control eating behavior

Ghrelin mediates its actions primarily through growth hormone secretagogue receptor-1a (GHS-R1a), stimulating the secretion of growth hormone. A chicken ghrelin receptor was discovered in different peripheral tissues, such as the pancreas, proventriculus, and also the brain, possibly suggesting autocrine/paracrine effects (Richards et al., 2006; Tanaka et al., 2003). Ghrelin receptor mRNA was detected in the hypothalamus (Chen et al., 2007; Sirotkin et al., 2013;

Song et al., 2018). However, ghrelin immunoreactivity was present in the chicken hypothalamus, its presence was not found in the IN nucleus (Ahmed and Harvey, 2002).

Food restriction was found to be able to increase ghrelin and GHS-R1a expression in the hypothalamus of layer hens, but the administration of ghrelin only resulted in a significant increase in GHS-R1a mRNA levels (Sirotkin et al., 2013). However, there was no significant effect of feeding restriction and refeeding on the hypothalamic expression of ghrelin and GHS-R1a in e broiler chickens (Chen et al., 2007).

Ghrelin transmits signals by binding to GHS-R1a and raising intracellular calcium levels. Chicken ghrelin was identified to elevate the intracellular calcium ion concentration in chicken cells (Tachibana et al., 2011). Ghrelin regulates feeding behavior in mammals through the AMPK signaling pathway. The interaction between ghrelin and AMPK was exerted through an increase in intracellular calcium levels and subsequent activation of CaMKKβ which in turn phosphorylated and activated AMPK (Andrews, 2011). In the case of chickens, central injection of ghrelin significantly inhibited AMPK subunits gene expression and phosphorylation of catalytic AMPK subunits in the hypothalamus. An inhibitory effect of ghrelin on the expression of CaMKKβ in chickens with low body weight but not high body weight chicks was also observed (Xu et al., 2011). Therefore, it has been proposed that AMPK signaling in the hypothalamus is responsible for the anorexigenic actions of ghrelin.

In mammals, ghrelin was shown to cause higher calcium levels via AMPK-mediated signaling that led to activation of ARC NPY neurons (Kohno et al., 2008). However, ghrelin administration did not affect hypothalamic NPY mRNA in neonatal layers. In addition, co-injection of ghrelin with NPY prevented the rise in feed intake that NPY causes (Saito et al., 2005).

Since ghrelin does not activate NPY neurons in the hypothalamus, it can be assumed that there is no orexigenic effect in chickens. Instead, it has been suggested that the inhibitory effect of ghrelin is mediated by the corticotropin-releasing hormone system, rather than through AgRP/NPY neurons. Ghrelin ICV administration activates the hypothalamic-pituitary-adrenal axis, resulting in higher plasma corticosterone levels (Saito et al., 2005). Furthermore, in support of this hypothesis, it was observed that vocalization, which is characteristic of hyperactivity behavior in chickens, significantly increased after ICV ghrelin injection. Herewith, similar behavior was also observed after injection of CRH, which in turn plays an important role in behavioral responses to stressors and in activation of the HPA axis (Saito et al., 2002b).

6. Conclusions and perspectives

Comparative analysis of the molecular mechanisms regulating feed intake has demonstrated that the majority of components and their interactions that orchestrate such complex biological processes in chickens are quite similar to their counterparts in mammals. In general, it can be suggested that the regulation of eating behavior is based on the integration of hormonal signals and nutritional status by the hypothalamus, which forms the state of satiety or hunger. The AMPK/mTOR signaling pathway, which is crucial to maintaining mammalian energy balance, is involved in the regulation of feeding behavior in chickens as well. However, there are conflicting effects of hormones on the regulation of feed intake in fast- and slow-growing chicken breeds. This is also confirmed by the heterogeneous results in the data on the expression of hypothalamic orexigenic and anorexigenic neuropeptides after hormone injection or feeding restriction. It seems that these differences are related to age, breed, period of food restriction, or source of the hormone used for the injection. However, further systems studies of the signaling pathways involved in

feed intake are required, with a focus on the role of hypothalamic neuropeptides in the formation of eating behavior. Moreover, the complex interrelationships between AMPK/mTOR and hormone-mediated signaling pathways with downstream regulation of neuropeptide expression cause the unintuitive dynamic behavior of the biological system. Therefore, an application of the mathematical modeling approach, including the development of detailed mechanistic and modular, spatially distributed models is pivotal for further investigation of the molecular mechanisms and their impact on feed intake and energy balance in chickens.

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Availability of data and materials

All conceptual diagrams in Systems Biology Graphical Notation (SBGN) standard reflecting signaling pathways and molecular mechanisms in Fig. 1 to 3 are available as a GitLab project at https://gitlab.sirius-web.org/collaboration/Chicken/Feed_intake. These diagrams can be considered as a growth point for further model development of a certain biological system regulating feed intake in chickens.

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Tables

Table 1. Neuropeptide central injections demonstrating effects on feeding behavior in chickens

Neuropeptide category	Neuropeptide	Type of chicken breed	Breed and age of chicken	Change in feed intake and references
Orexigenic neuropeptide	NPY	Broiler	2- and 3-d-old Cobb	Increased (Ando et al., 2001)
			2-, 4- and 8-d-old chunky	Increased (Saneyasu et al., 2011b)
		Layer	6-d-old	Increased (Tachibana et al., 2006)
			2-, 4- and 8-d-old White Leghorn	Increased (Saneyasu et al., 2011b)
	AgRP	Broiler	3-d-old Cobb	No change (Tachibana et al., 2001)
		Layer	4-d-old Boris Brown	Increased (Tachibana et al., 2001)
Anorexigenic neuropeptide	POMC (α-MSH)	Broiler	5-d-old Cobb 500	Decreased (Cline and Smith, 2007)
			8-d-old chunky	Decreased (Honda et al., 2012)
			2-d-old Cobb	Decreased (Kawakami et al., 2000)
			1-d-old chunky	Decreased (Saneyasu et al., 2011a)
		Layer	8-d-old White Leghorn	Decreased (Honda et al., 2007, 2012)
			6-d-old	Decreased (Tachibana et al., 2007)

NPY = neuropeptide Y; AgRP = agouti-related peptide; POMC = proopiomelanocortin; α-MSH = α-melanocyte-stimulating hormone.

1025 Table 2. Changes in expression of neuropeptides in the hypothalamus of feed-restricted chickens

Neuropeptide category	Neuropeptide	Type of chicken breed	Breed and age of chicken	Fasting period	Change in neuropeptide expression and references
Orexigenic neuropeptide	NPY	Broiler	14-d-old yellow-feathered	48 and 24 h	Increased (Fang et al., 2014)
			7-d-old Arbor Acres	48 h	Increased (Song et al., 2012)
			1-d-old Ross × Cobb	48 h	Increased (Higgins et al., 2010)
			7-d-old Arbor Acres	24 h	No change (Liu and Zhu, 2012)
			21-d-old Ross 308	12 h	Increased (Kewan et al., 2021)
			6-wk-old Ross 308	Chronic feed restriction for 6 wk	No change (Dunn et al., 2013)
		Layer	21-d-old White Leghorn	12 h	Increased (Kewan et al., 2021)
			10-d-old White Leghorn	4 d	Increased (Kameda et al., 2001)
	AgRP	Broiler	14-d-old yellow-feathered	48 and 24 h	Increased (Fang et al., 2014)
			7-d-old Arbor Acres	48 h	Increased (Liu and Zhu, 2012)

		7-d-old Arbor Acres	48 h	Increased (Song et al., 2012)
		1-d-old Ross × Cobb	48 h	Increased (Higgins et al., 2010)
		21-d-old Ross 308	12 h	Increased (Kewan et al., 2021)
		6-wk-old Ross 308	Chronic feed restriction for 6 wk	Increased (Dunn et al., 2013)
	Layer	21-d-old White Leghorn	12 h	No change (Kewan et al., 2021)

NPY = neuropeptide Y; AgRP = agouti-related peptide.

1028 Table 3. Effects of hormone injection on feeding behavior
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Hormone	Type of chicken breed	Breed and age of chicken	Change in feed intake and references
Insulin	Broiler	20-d-old Ross 308	Decreased (Yousefvand et al., 2018)
		4-d-old Chunky	No change (Shiraishi et al., 2011b)
		5-d-old	Decreased (Yousefvand et al., 2020)
	Layer	8-d-old White Leghorn	Decreased (Honda et al., 2007)
		3- or 4-d-old Single Comb White Leghorn	Decreased (Shiraishi et al., 2008, 2009, 2011b)
Leptin	Broiler	4-d-old Hubbard × Cobb-500	No change (Sims et al., 2017)
		7-wk-old	Decreased (Denbow et al., 2000)
	Layer	4-wk-old Single Comb White Leghorn	Decreased (Denbow et al., 2000)
		12-wk-old White Rock high body weight line	No change (Kuo et al., 2005)
		12-wk-old White Rock low body weight line	Decreased (Kuo et al., 2005)

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1031 Table 4. Effects of hormone injection on the expression of hypothalamic neuropeptides

Hormone	Type of chicken breed	Neuropeptide	Breed and age of chicken	Change in neuropeptide expression and references
Insulin	Broiler	NPY	-	-
		AgRP	-	-
		POMC	7-d-old Ross 308	No change (Saneyasu et al., 2019)
	Layer	NPY	3- or 4-d-old Single Comb White Leghorn	Decreased (Shiraishi et al., 2008)
			8-d-old White Leghorn	No change (Honda et al., 2007)
		AgRP	3- or 4-d-old Single Comb White Leghorn	No change (Shiraishi et al., 2008)
			8-d-old White Leghorn	No change (Honda et al., 2007)
		POMC	3- or 4-d-old Single Comb White Leghorn	Increased (Shiraishi et al., 2008)
			8-d-old White Leghorn	Increased (Honda et al., 2007)
			7-d-old White Leghorn	Increased (Saneyasu et al., 2019)
Leptin	Broiler	NPY	3-wk-old Ross	Decreased (Dridi et al., 2005)
		AgRP	3-wk-old Ross	No change (Dridi et al., 2005)

	POMC	3-wk-old Ross	No change (Dridi et al., 2005)
Layer	NPY	-	-
	AgRP	-	-
	POMC	-	-

1032 NPY = neuropeptide Y; AgRP = agouti-related peptide; POMC = proopiomelanocortin

Figures

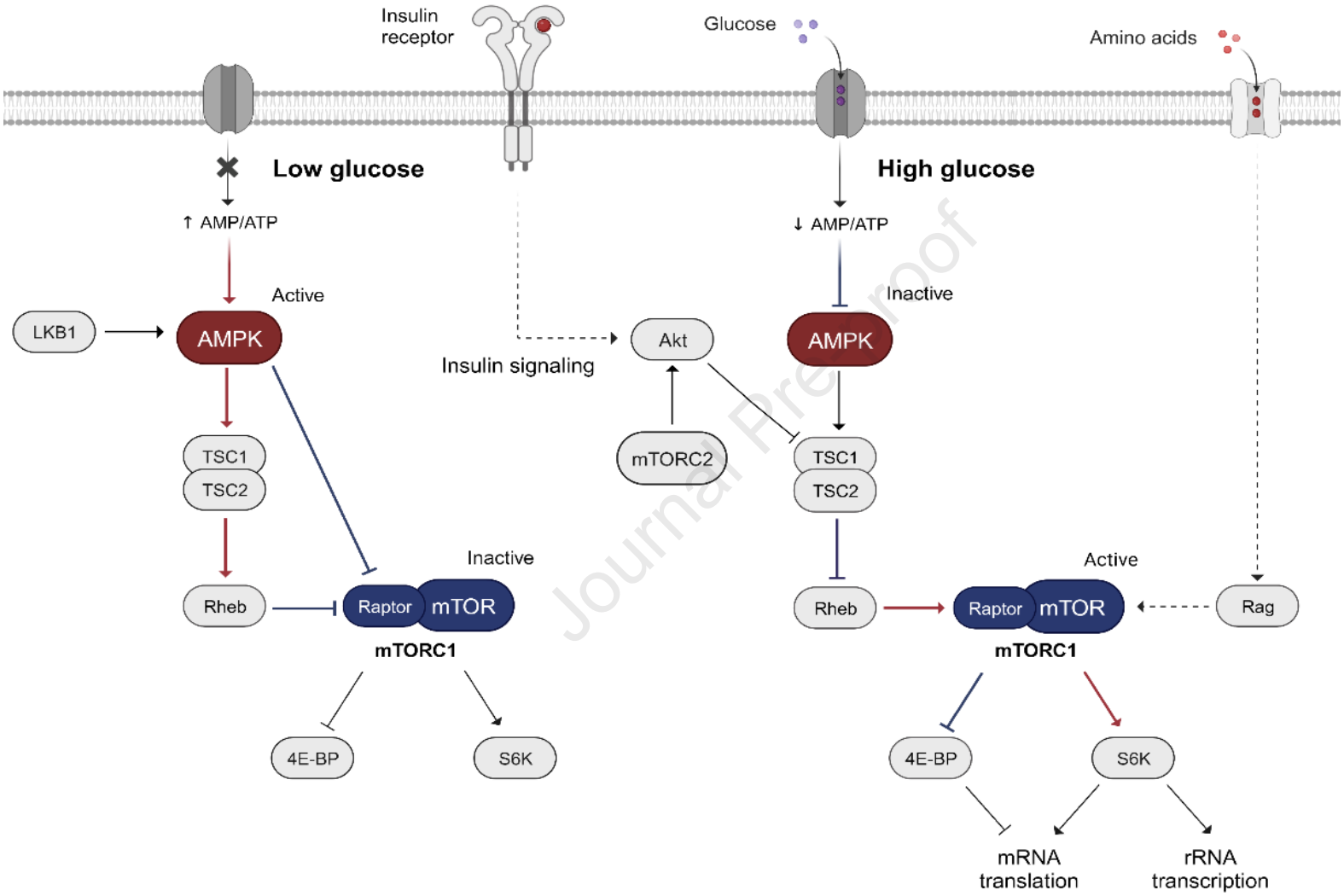

Fig. 1 AMPK/mTOR signaling pathway involved in regulation of feed intake in chickens (created with BioRender.com). AMP = adenosine monophosphate; ATP = adenosine triphosphate; LKB1 = liver kinase B1; AMPK = adenosine-monophosphate activated protein kinase;

TSC1/TSC2 = tuberous sclerosis complex; Rheb = Ras homologue enriched in brain; mTOR = mammalian target of rapamycin; Raptor = regulatory-associated protein of mTOR; 4E-BP = 4E-binding protein 1; S6K = S6 kinase; Akt = protein kinase B; mTORC1 = mTOR complex 1; mTORC2 = mTOR complex 2; Rag = recombination-activating gene protein.

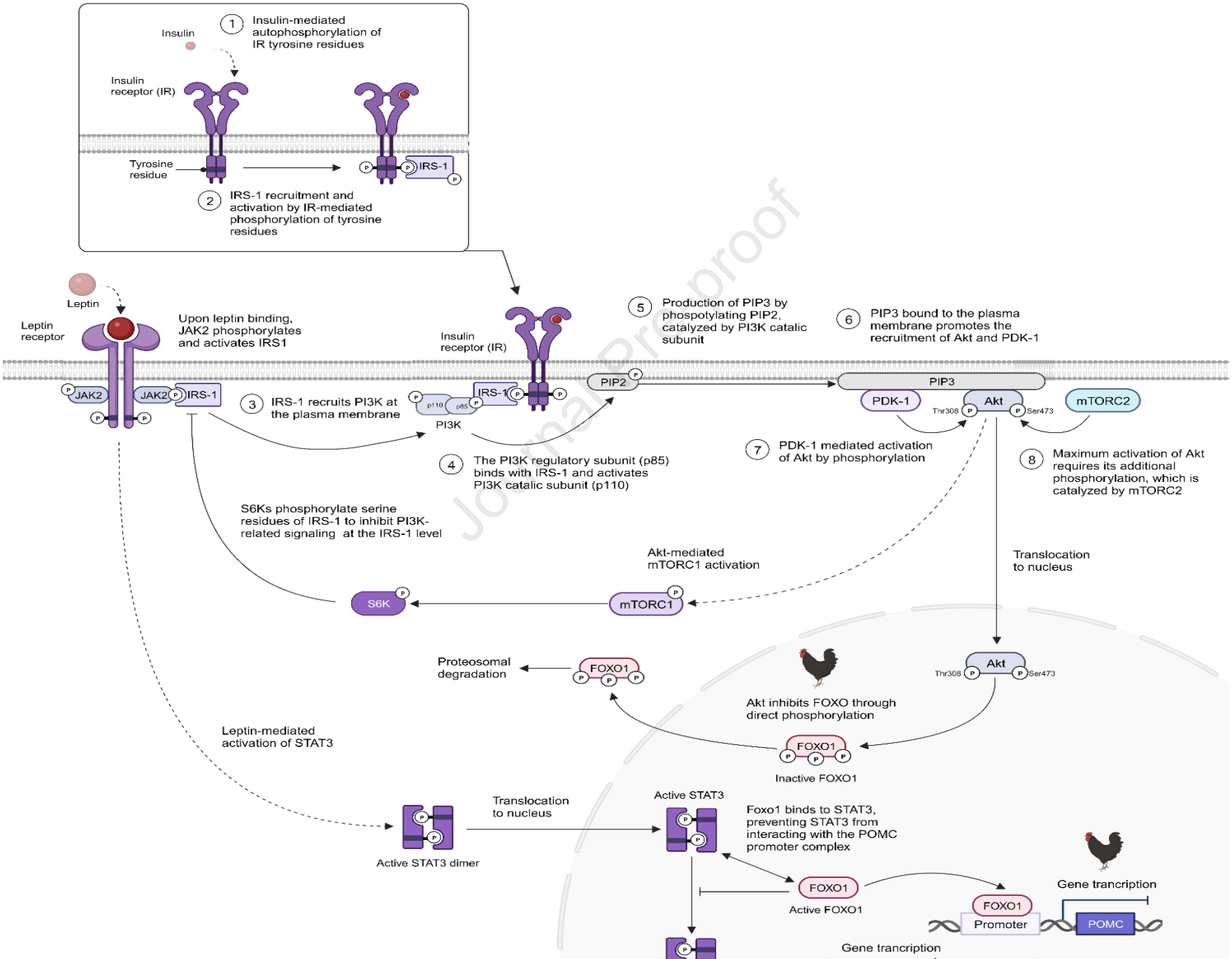

Fig. 2 Overview of leptin and insulin signaling pathways in the hypothalamus of chickens regulating neuropeptide genes (created with BioRender.com). The mechanism of insulin action in chickens may differ from what is known in mammals. FOXO1 signaling is significantly different between broilers and laying hens. In broilers, there is a slight phosphorylation of FOXO1 and no significant change in the expression of the anorexigenic neuropeptide POMC. While the opposite pattern is shown for laying hens, which is similar to the characteristic of mammals. Note: Pattern of a chicken indicates differences in signaling pathway compared to mammals. IR = insulin receptor; IRS-1 = insulin receptor substrate 1; JAK2 = Janus tyrosine kinase 2; PI3K = phosphoinositide 3-kinase; PIP2 = phosphatidylinositol 4,5-bisphosphate; PIP3 = phosphatidylinositol (3,4,5)-triphosphate; PDK-1 = phosphoinositide-dependent protein kinase 1; Akt = protein kinase B; mTORC1 = mammalian target of rapamycin complex 1; mTORC2 = target of rapamycin complex 2; S6K = S6 kinase; FOXO1 = forkhead box protein O1; STAT3 = signal transducer and transcription 3; POMC = proopiomelanocortin; SOCS3 = suppressor of cytokine signaling 3; NPY = neuropeptide Y.

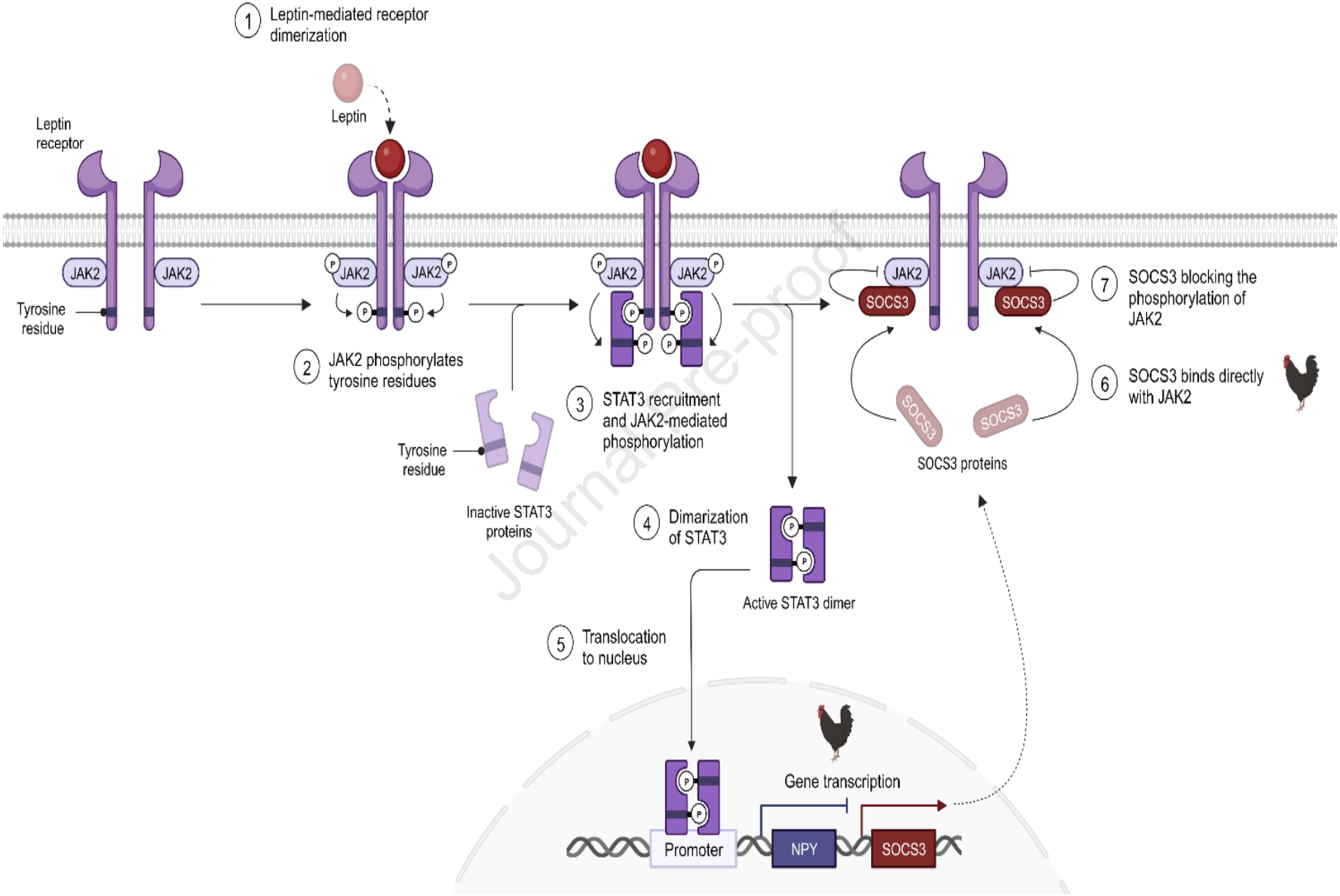

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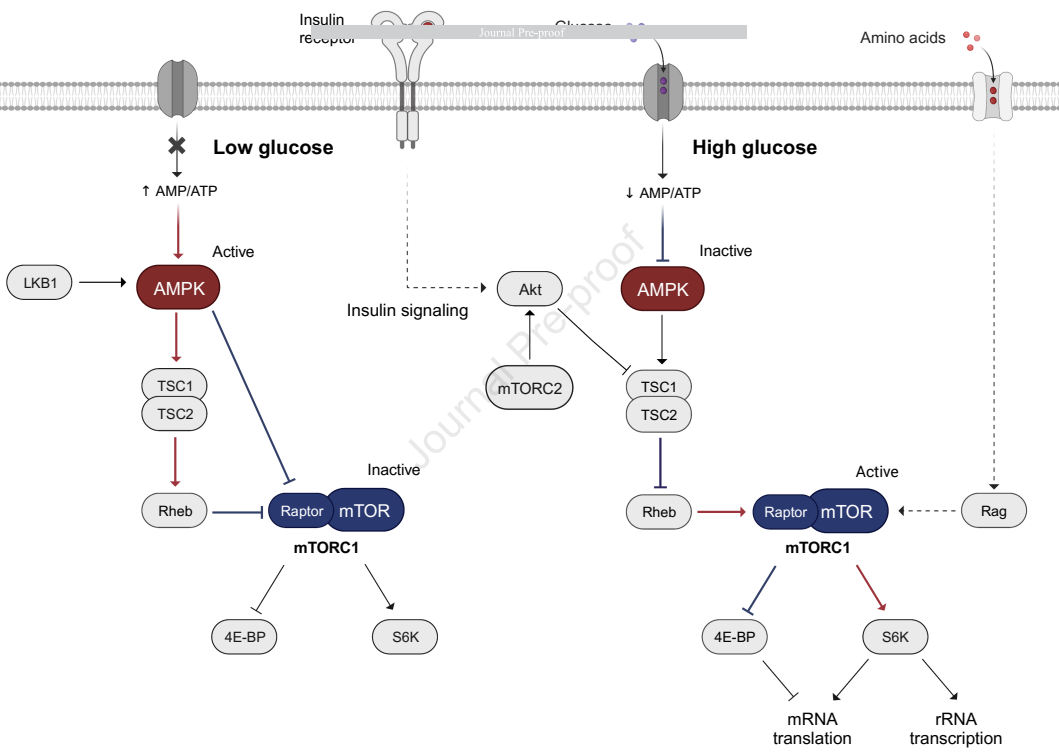
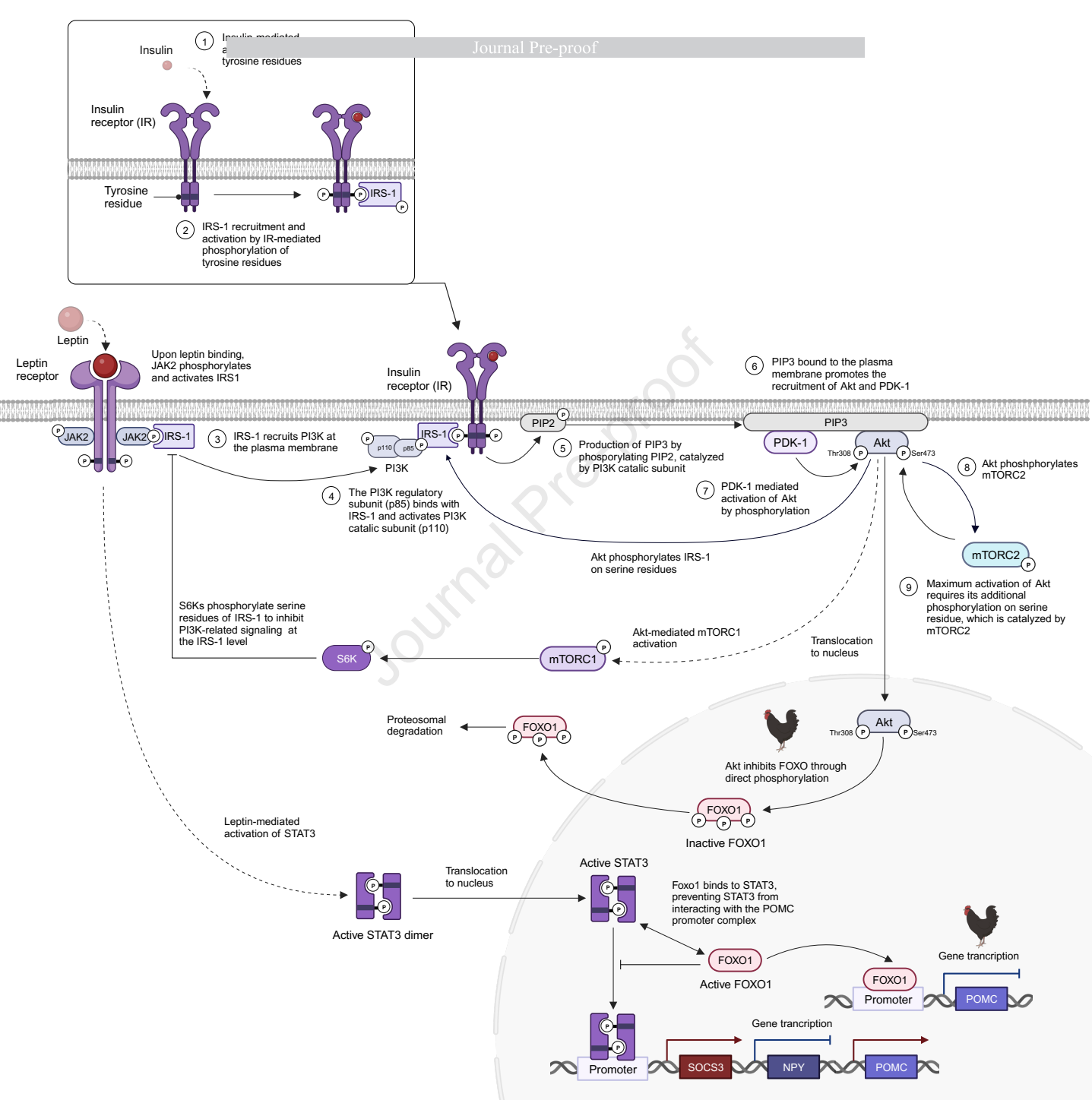
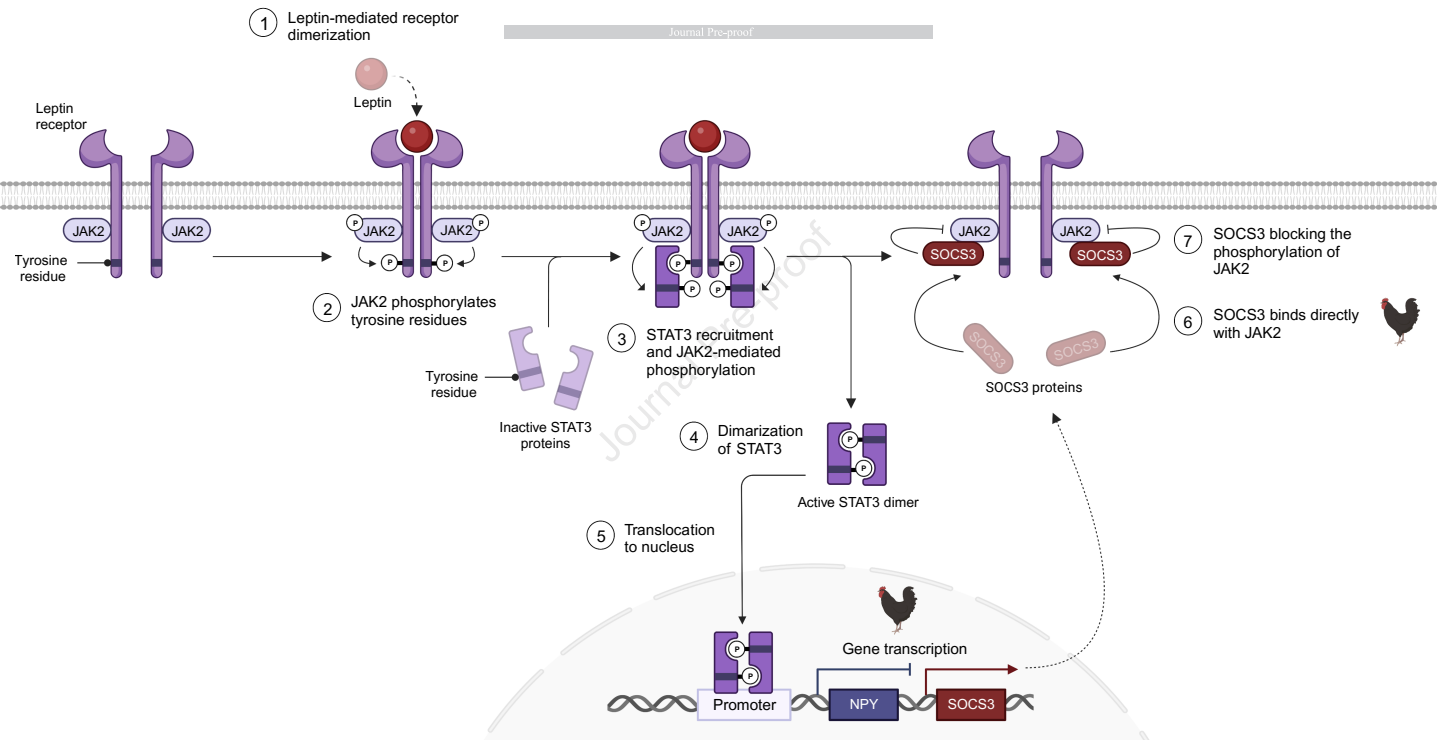
Fig. 3 Detailed leptin signaling pathway in the hypothalamus of chickens (adapted from “Cytokine Signaling through the JAK-STAT Pathway”, by BioRender.com (2023). Retrieved from https://app.biorender.com/biorender-templates). The mechanism of leptin action in chickens may differ from what is known in mammals. In SOCS3-mediated inhibition of leptin signaling, chicken SOCS3 probably does not interact with p-Tyr986 in the intracellular domain of chicken LEPR, but directly binds to JAK2. In contrast to mammals, leptin is more likely to affect feeding behavior of chickens through the expression of the orexigenic neuropeptide NPY (but not AgRP), without affecting the expression of anorexigenic POMC. Note: The pattern of a chicken indicates differences in signaling pathway compared to mammals. LEPR = leptin receptor; JAK2 = Janus tyrosine kinase 2; STAT3 = signal transducer and transcription 3; SOCS3 = suppressor of cytokine signaling 3; NPY = neuropeptide Y.







Declaration of conflict of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: