Scientific Reports
https://doi.org/10.1038/s41598-025-32547-0
Article in Press

Arginine shapes growth and mTOR pathway gene expression in Japanese quails (*Coturnix japonica*)

Received: 15 October 2025
Accepted: 10 December 2025
Published online: 17 December 2025

Cite this article as: Gashew M., Reda G.K., Almira F.N. *et al.* Arginine shapes growth and mTOR pathway gene expression in Japanese quails (*Coturnix japonica*). *Sci Rep* (2025). https://doi.org/10.1038/s41598-025-32547-0

Mequanint Gashew, Gebrehaweria K. Reda, Fadella Nur Almira, Eman Moustafa Abdelbary, Doha Mohamad Khalifeh, Sawadi F. Ndunguru, Gabriella Gulyás, Renáta Knop, Brigitta Csernus, Csaba Szabó, Ádám Z. Lendvai & Levente Czeglédi

We are providing an unedited version of this manuscript to give early access to its findings. Before final publication, the manuscript will undergo further editing. Please note there may be errors present which affect the content, and all legal disclaimers apply.

If this paper is publishing under a Transparent Peer Review model then Peer Review reports will publish with the final article.

ARTICLE IN PRESS

© The Author(s) 2025. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

ARTICLE IN PRESS

Arginine shapes growth and mTOR pathway gene expression in Japanese quails (*Coturnix japonica*)

Mequanint Gashew[1,2,5,*], Gebrehaweria K. Reda[1,2,3], Fadella Nur Almira[1,2], Eman Moustafa Abdelbary.[1,2], Doha Mohamad Khalifeh[1,2], Sawadi F. Ndunguru[1,2,3], Gabriella Gulyás[1], Renáta Knop[1], Brigitta Csernus[3], Csaba Szabó[4], Ádám Z. Lendvai[3,+], Levente Czeglédi[1,+]

[1]Department of Animal Science, Institute of Animal Science, Biotechnology and Natural Conservation, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen, 4032 Debrecen, Hungary
[2] Doctoral School of Animal Science, University of Debrecen, 4032 Debrecen, Hungary
[3]Department of Evolutionary Zoology and Human Biology, University of Debrecen, 4032 Debrecen, Hungary
[4]Department of Animal Nutrition and Physiology, Faculty of Agriculture and Food Sciences and Environmental Management, University of Debrecen, Debrecen 4032, Hungary
[5]Department of Animal Science, Agriculture and Environmental Science College, Debre Tabor University, 272 Debre Tabor, Ethiopia

* Corresponding author: mgashaw933@gmail.com

[+] These authors contributed equally to the study.

ARTICLE IN PRESS

ABSTRACT

Arginine is a crucial amino acid that regulates growth, metabolism, and cellular homeostasis through nutrient-sensing pathways such as the mechanistic target of rapamycin (mTOR). However, the mechanisms by which dietary arginine modulates mTOR signalling and associated gene networks during early development remain poorly understood in birds. Here, we tested the effects of dietary arginine levels (low, control, and high) on body mass, gene expression, and gene network connectivity in Japanese quail chicks (*Coturnix japonica*). Using 153 birds over a 14-day experiment, we show that post-hatch arginine restriction significantly impairs growth in both sexes, while gene expression and gene network connectivity responses to arginine availability are strongly sex-specific. Arginine supplementation in females led to coordinated upregulation of

ARTICLE IN PRESS

growth-related genes (*IGF1*, *GHR*), translation regulators (*EIF4EBP1*), lipid metabolism genes (*FASN, FABP1*), and oxidative defence (*SOD2*). In contrast, males showed more limited changes, primarily involving *MTOR* and autophagy-related gene *ATG5*. Gene network analysis revealed enhanced connectivity and centrality in females under arginine supplementation, suggesting a more extensive gene interaction response, while males showed sparser networks under restriction, with *MTOR* and *IGF1* emerging as key regulatory hubs. These findings provide novel insights into the sensitivity of developing birds to early nutritional conditions and suggest that the mechanisms underlying growth, gene expression, and gene networking can differ between the sexes, even without sex differences in phenotypic traits. Our results highlight the importance of considering sex-specific gene regulatory architectures when evaluating nutritional effects on vertebrate development.

Keywords: Amino acid, gene expression, growth, poultry, mechanistic target of rapamycin

ARTICLE IN PRESS

INTRODUCTION

Nutrition is a fundamental determinant of organismal development, shaping growth trajectories, metabolic efficiency, reproduction, and long-term health [1]. In the postnatal period, the availability of specific nutrients can act as a cue that calibrates gene expression, resource allocation, and developmental priorities, a phenomenon referred to as nutritional programming [2]. Amino acids, beyond their role as protein building blocks, serve as potent signalling molecules that activate nutrient-sensitive pathways regulating growth, immunity, metabolism, and cellular stress responses [3].

One of the central signalling hubs linking nutrient status to anabolic processes is the mechanistic target of rapamycin (mTOR) signalling pathway, activated by specific amino acids, especially leucine, methionine, and arginine [4–6]. The mTOR pathway integrates signals from nutrients, growth factors, and cellular energy levels to regulate protein synthesis and cell growth through downstream effectors such as eukaryotic translation initiation factor 4E binding protein 1 (4EBP1) and autophagy gene [7]. This molecular pathway is highly conserved across animals and provides a mechanistic framework for understanding how diet influences physiology and performance [8].

ARTICLE IN PRESS

Among mTOR-regulating amino acids, arginine plays a particularly multifaceted role. Some vertebrate taxa, including birds and reptiles, have an incomplete urea cycle and cannot synthesise arginine endogenously, making its dietary intake critical [9,10]. It also serves as a precursor for nitric oxide, creatine, and polyamines, linking it to vascular function, immune competence, and nutrient absorption [11,12]. Arginine availability modulates mTOR activity and downstream gene expression in mammals, fish, and birds [13,14], with both deficiency and excess eliciting distinct, dose and context-dependent effects on growth and metabolism [13–16].

Sex-specific responses to nutritional signals are increasingly recognised as key components of developmental plasticity [17]. Males and females often diverge in their growth strategies, reproductive allocation, and metabolic priorities, especially during critical developmental windows when nutrients are scarce or imbalanced [18]. Research on fish, mice, and Japanese quail demonstrates that males and females exhibit distinct gene expression patterns in response to dietary and environmental factors, particularly in genes associated with mTOR and autophagy signalling [19–21]. These differences are influenced by sex hormones and body size, highlighting the importance of considering both factors when studying growth and metabolic regulation [20,22].

Birds offer unique and underutilised opportunities for understanding the evolutionary basis of physiological plasticity. Their uricotelic nitrogen excretion, high metabolic rate, and rapid development present a contrast with mammalian models and enable comparative insights into how nutrient-sensing pathways like mTOR evolve and function under divergent life-history constraints. This makes avian systems particularly suitable for disentangling the roles of nutrient availability, sex, and developmental stage in shaping gene expression responses. Importantly, findings from bird models are increasingly being recognised for their relevance to broader ecological and evolutionary questions, as well as for informing biomedical research in areas like ageing, oxidative stress, and metabolic disease [23].

In this context, we used the Japanese quail (*Coturnix japonica*) as a model to explore how dietary arginine availability during early post-hatch development influences body mass and hepatic expression of genes related to the mTOR pathway, lipid metabolism, autophagy, and oxidative stress. Quails are widely used in poultry research and serve as a tractable vertebrate model for developmental and molecular physiology due to their short generation time and well-characterised developmental stages [24,25]. While earlier studies have linked arginine availability to mTOR activation in chicken [26], it remains poorly understood how dietary arginine orchestrates

ARTICLE IN PRESS

coordinated gene regulatory responses during early post-hatch development, particularly in relation to growth, metabolism, and oxidative balance. Our study addresses this gap by mapping how dietary arginine modulates these interconnected pathways in a growing vertebrate.

To investigate how arginine modulates metabolic and growth-related pathways, we examined a panel of genes representing distinct but interconnected physiological functions which represented core regulatory nodes of the mTOR pathway and its principal downstream processes [6,20,27–29]. These genes provide an integrated view of how dietary arginine influences growth, metabolism, and stress responses through the mTOR pathway. *MTOR* and its downstream target, *EIF4EBP1* (Eukaryotic translation initiation factor 4E binding protein), were included as core regulators of nutrient sensing and protein synthesis. Lipid metabolism was assessed through *FASN* (Fatty acid synthase), which drives *de novo* fatty acid synthesis, and *FABP1* (Fatty acid binding protein 1), which facilitates intracellular fatty acid transport and utilisation. *FASN* and *FABP1* were chosen as functional effectors of lipid synthesis and transport, both of which are highly responsive to dietary amino acid supply in avian species [30–32]. We assessed autophagy through *ATG5* (Autophagy-related 5), a key mediator of autophagosome formation during nutrient stress. Oxidative balance was gauged via *SOD2 (*Superoxide dismutase 2*)*, a mitochondrial antioxidant enzyme involved in neutralising reactive oxygen species. *SOD2* is localised mainly in mitochondria, the primary site of reactive oxygen species generation under nutrient and metabolic stress, where it protects the electron transport chain from oxidative damage caused by superoxide radicals [33–35]. Finally, we included *IGF1(*insulin-like growth factor 1) and its upstream *GHR (*growth hormone receptor), central components of the somatotropic axis that regulate systemic growth and hepatic metabolism. Collectively, these genes provide insight into how dietary arginine shapes coordinated molecular responses related to growth, energy allocation, cellular maintenance, and stress resilience during early development.

Given the liver's central role in nutrient sensing and arginine activation of the *mTOR* pathway, we hypothesised that dietary arginine levels modulate body mass and hepatic expression of *mTOR*-related genes in growing Japanese quails. Thus, by experimentally manipulating dietary arginine levels (low, control, and high) and analysing gene expression across eight functionally related genes in males and females, we aimed to test how early nutritional signals are integrated at the molecular level, and whether this integration differs between sexes.

ARTICLE IN PRESS

MATERIAL AND METHODS

Experimental animal management

The experiment was conducted at the Animal House of the Institute of Animal Science, Biotechnology, and Nature Conservation, University of Debrecen, Hungary. We collected 500 eggs from 95 female Japanese quails over five days and incubated them artificially in an industrial incubator (WQ-63 Model 2021 Version 2, AGROFORTEL, Budapest, Hungary) under standard conditions [28] About 350 healthy hatchlings were obtained and reared with *ad libitum* access to feed and water until they reached two weeks of age. Infrared lamps maintained the initial rearing temperature of 37 °C for four days. After that, it was lowered by 3 °C every four days until it reached 24 °C at the end of the two weeks. Relative humidity in the cage was from 60 to 65%. After the experiment started, the room was maintained at 24 ± 3°C, 60–75% relative humidity, a 12:12 h light-dark cycle, and 20 lux light intensity [36]. The quails were fed a grower diet formulated based on corn, wheat, corn germ meal, corn gluten meal, and fishmeal, containing 22% crude protein and 12.13 MJ/kg metabolizable energy (Table 1).

ARTICLE IN PRESS

Table 1. Feed composition and calculated nutrient content of the experimental diets.

Ingredients	Treatments		
	Control	Low arginine	High arginine
Corn	3.40	3.40	3.40
Wheat	30.00	30.00	30.00
Corn germ meal	39.19	39.19	39.19
Corn gluten meal	10.90	10.90	10.90
Fishmeal	3.74	3.74	3.74
Oil	8.08	8.08	8.08
Limestone	1.40	1.40	1.40
MCP	0.42	0.42	0.42
L-Lys	0.77	0.77	0.77
DL-Met	0.06	0.06	0.06
L-Thr	0.28	0.28	0.28
L-Trp	0.06	0.06	0.06
L-Ile	0.20	0.20	0.20
L-Arg	0.39	0	0.78
Inert (kaolin)	0.39	0.78	0
Salt	0.22	0.22	0.22
Premix[a]	0.50	0.50	0.50
Nutrient content (%)			
ME (MJ/kg)	12.13	12.13	12.13
Crude protein	22.00	22.00	22.00

Lys	1.30	1.30	1.30
Met	0.50	0.50	0.50
Thr	1.02	1.02	1.02
Trp	0.22	0.22	0.22
Leu	2.16	2.16	2.16
Ile	0.98	0.98	0.98
Arg	1.25	0.94	1.56
Leu/Ile	2.20	2.20	2.20
Ca	0.80	0.80	0.80
P	0.56	0.56	0.56
non phytate P	0.30	0.30	0.30
Na	0.15	0.15	0.15

[a] The premix provided the following per kilogram of complete diet: 5000 NE vitamin A, 1000 NE vitamin D_3, 24.5 mg/kg vitamin E, 1 mg vitamin K_3, 0.75 mg vitamin B_1, 2.5 mg vitamin B2, 6 mg Ca-d-Pantothetane, 2 mg vitamin B_6, 10 ug vitamin B_{12}, 55 µg biotin, 12.5 mg niacin, 0.3 mg folic acid, 1500 mg choline chloride, 66 mg Zn, 9.6 mg Cu, 48.1 mg Fe, 66 mg Mn, 0.9 mg I, 0.21 mg Se, 60 µg Co. MCP: monocalcium phosphate; ME: metabolisable energy

ARTICLE IN PRESS

Experimental design and sampling

At two weeks of age, 153 unsexed, similar-sized Japanese quails were selected and randomly distributed to three treatment groups. The experiment followed a completely randomised design with three treatments and three replicates, each containing seventeen birds. The dietary treatments included a control group: a diet formulated per [37], a high arginine group (control diet supplemented with 25% arginine above the recommended amount (1.56%)), and a low arginine group (control diet restricted by 25% of arginine from the recommended amount (0.94%)) (Table 1). The restriction or supplementation of arginine from the recommended level provides a balance between biological relevance and animal welfare by imposing a measurable nutritional stress without causing gross toxicity or severe deficiency. Several recent studies in poultry have used ±20–35% changes to investigate functional nutrients and amino acid effects [20,27,29,38–40]. Quails were housed in groups in identical cages (40 cm × 50 cm × 40 cm; length × width × height) within the same facility to ensure consistent environmental conditions and were tagged individually with numbered plastic rings for individual body mass measurement. Feed was provided in specially designed feeders to minimise spillage. The trial was conducted for 14 days [27]. Daily fresh feed was replaced between 08:00 and 09:00, and feed intake was recorded. The average daily feed intake was 13.53 ± 0.16g for low arginine, 14.47± 0.41g for control, and 14.65 ± 0.49g for high arginine

ARTICLE IN PRESS

groups. Feed intake was lowered in the low arginine groups compared with other groups. Live body weight was measured using a digital balance (± 0.1 g accuracy) on days 0 (initial), 7 (midpoint), and 14 (final) to assess body mass across treatments.

On day 14 of the trial, 48 birds (eight per treatment group of both male and female) were euthanised by cervical dislocation following sedation with midazolam (5 mg/mL; EGIS Pharmaceuticals PLC, Hungary) performed by professional veterinarians for tissue collection. Liver tissues were immediately collected, placed in sterile tubes, flash-frozen, transported to the laboratory, and stored at −80°C until analysis.

RNA extraction and purification

Total RNA was extracted from liver tissues using the peqGOLD Total RNA Kit (VWR, USA) with on-column DNase I digestion, following the manufacturer's instructions. RNA concentration and purity were assessed using a Synergy HT Multi-mode Microplate Reader (BioTek Instruments, Winooski, VT, USA). Integrity was checked with a Qubit 4 fluorometer (Invitrogen™), and it was in the range of 7-10.

Complementary DNA (cDNA) was synthesised in a PCRmax AlphaThermal Cycler (Cole-Parmer Ltd., Vernon Hills, IL, USA) using the LunaScript® RT SuperMix Kit (New England Biolabs Inc., Ipswich, MA, USA) with 800 ng RNA, 5x LunaScript RT Supermix, and nuclease-free water in a 20 µL final volume. Thermal conditions included 25°C for 2 min (annealing), 55°C for 10 min (reverse transcription), and 95°C for 1 min (inactivation). cDNA was diluted 10-fold and stored at −20°C for qPCR.

ARTICLE IN PRESS

Quail-specific, intron-spanning primers were designed using Oligo 7 software (version 7.6) and checked for target identity via the Primer BLAST web based tool (NCBI, https://www.ncbi.nlm.nih.gov/tools/primer-blast/, accessed on January 28, 2025) (Table 2). qPCR was performed with AriaMx Real-Time PCR System (Agilent Technologies, Santa Clara, CA, USA) using HOT FIREPol® EvaGreen® qPCR Mix Plus (Solis BioDyne, Teaduspargi, Estonia) according to the manufacturer's protocol. Each reaction included 5x HOT FIREPol® EvaGreen® qPCR Mix Plus Solis BioDyne, Teaduspargi, Estonia, 8 ng cDNA template, 200 nM of each primer, and distilled water in a 10 µL final volume. Reactions were run in duplicates using 96-well plates (Sorenson 2633), with calibrators included to control for interplate variation, and no template controls were included for each primer. Cycling conditions were 95°C for 12 min, followed by 40 cycles of 95°C for 15 s, 60°C for 20 s, and 75°C for 20 s, with melt curve analysis to confirm specificity.

ARTICLE IN PRESS

Table 2. Primer design for the target and reference gene

Gene	Primer sequences (5´ < ---- > 3´) Forward/reverse	Gene bank accession no.	Amplicon Length (bp)	Melting temperature (Tm)
MTOR	F: CCG AAG CAT TGA ATT GGC CCT R: CAT CTC TCA AAG GCA GCG GAC C	XM_015882433.2	116	F:61.57 R: 63.50
IGF1	F: CAC TAT GCG GTG CTG AGC TGG TT R: ATC CCC TTG TGG TGT AAG CGT CT	XM_015867574.2	118	F: 65.42 R: 63.80
GHR	F: GGC ACT GGT CTG TGT GAA TGA CT R: CCA GCT CAG GTG ATC TGC ACT T	XM_032441512.1	89	F: 62.93 R: 62.58
FASN	F: TCA GCC CGA ACC TCC GCC AT R: ATG CCT GCA ATC ACC ACG TCT	XM_015879647.2	72	F: 66.24 R: 62.67
FABP1	F: AGT CCC ATG AGA ACT TTG AGC CTT R: CTG GAT CTG TTC ATC AGG AAG CCC	XM_015862881.2	62	F: 62.04 R: 63.03
EIF4EBP1	F: ACC AGC CCA ATT GTG GAG GAG TT R: CTC AGG GCA CGT GCT TTA GAT GT	XM_015883175.1	120	F: 64.29 R: 63.03
ATG5	F: ATA GTG GAT TTC GGT ACA TCC CA R: TCC TCC AGA AGC AAT TGG TCG	XM_015858735.1	95	F: 59.03 R: 60.34
SOD2	F: ACA GCA AAC ACC ACG CCA CCT R: AGC GAC ACC TGA GCT GTA ACA TC	XM_015858046.1	100	F: 65.38 R: 62.77
RPL19	F: CAT CGG TAA GAG GAA GGG T R: ACG TTG CCC TTG ACC TTC AG	XM_015885843.1	162	F: 55.80 R: 60.54

Abbreviations: *MTOR*, Mechanistic target of rapamycin; *IGF1*, Insulin-like growth factor 1; *GHR*, Growth hormone receptor; *FASN*, Fatty acid synthase; *FABP1*, Fatty acid binding protein 1; *EIF4EBP1*, Eukaryotic translation initiation factor 4E binding protein; *ATG5*, Autophagy-related 5; *SOD2*. Superoxide dismutase 2; *RPL19*, Ribosomal protein L19

Reference gene stability was assessed for *RPL19*, *GAPDH*, and *RN18S* using NormFinder, BestKeeper, and Delta Ct algorithms. *RPL19* was identified as the most stable and used for normalisation [41,42]. Relative expression of mechanistic target of rapamycin (*MTOR*), insulin-like growth factor 1 (*IGF1*), growth hormone receptor (*GHR*), fatty acid synthase (*FASN*), fatty acid binding protein 1 (*FABP1*), eukaryotic translation initiation factor 4E binding protein 1 (*EIF4EBP1*), autophagy-related 5 (*ATG5*), and antioxidant gene superoxide dismutase (*SOD2*) was quantified as fold change using the $2^{-\Delta\Delta Ct}$ method [43]. Log-transformed expression values were used for statistical analysis.

ARTICLE IN PRESS

Statistical analysis

All statistical analyses were performed using R v. 4.2.2 [44]. Data visualisation was conducted using the 'ggplot2' package (version 3.4.3).

To evaluate the effects of treatment, sex, and their interaction on body mass over the 14-day trial period, we fitted linear mixed models (LMMs) using the 'lmer' function from the 'lme4' package [45]. Treatment, sex, and time effects were included as fixed factors, while individual cages were treated as random effects. The best-fitting model was selected using Akaike's Information Criterion corrected (AICc) [46]. The significance of fixed effects was assessed using the 'lmerTest' package (version 3.1.3) to compute p-values using two-way ANOVA [47]. Post-hoc pairwise comparisons of means were performed using estimated marginal means (via the 'emmeans' package) with Tukey's HSD test at a significance level of $p < 0.05$ [47,48]. For gene expression analysis, linear models were employed to examine the effects of treatment, sex, and their interaction on the expression of target genes. In a gene network connectivity analysis, degree centrality measures how many direct connections a gene has, emphasising the importance of connections with high-degree genes (hubs) to the overall network function. Betweenness centrality reflects a gene's role as a bridge, indicating how often it lies on the shortest paths between other genes. In contrast, closeness centrality assesses how close a gene is to all other genes in the network, providing insight into the efficiency of information flow through that gene. P-values were calculated for the Benjamini–Hochberg False Discovery Rate (FDR) method. However, gene co-expression networks were constructed based on correlation strength rather than statistical significance. In this network, genes are represented as nodes, and nodes are connected when there is a high level of co-expression between the corresponding genes [49] and using the igraph package in R.

Ethical approval

The experiment was conducted in compliance with the EU Directive 2010/63/EU on the protection of animals used for scientific purposes. Approval was obtained from the Ethical Committee for Animal Use at the University of Debrecen, Hungary (Protocol No. 5/2021/DEMAB). All procedures adhered to institutional guidelines and regulations. The reporting of this study follows the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines.

ARTICLE IN PRESS

RESULTS

Arginine restriction reduces body mass in both sexes

Arginine restriction led to a significant, time-dependent reduction in body mass in both male (treatment × time: $F_{4, 134}$ = p <0.001) and female (treatment × time: $F_{4,156}$ = 10.72, p < 0,001) quails. During the first week, the treatments had no effect on body mass. However, by the second week, birds on the low-arginine (LA) diet weighed less than those in the control and high-arginine (HA) groups (females: all p < 0.001, Fig. 1a; males: LA vs control: p = 0.003, LA vs HA: p < 0.001, Fig. 1b).

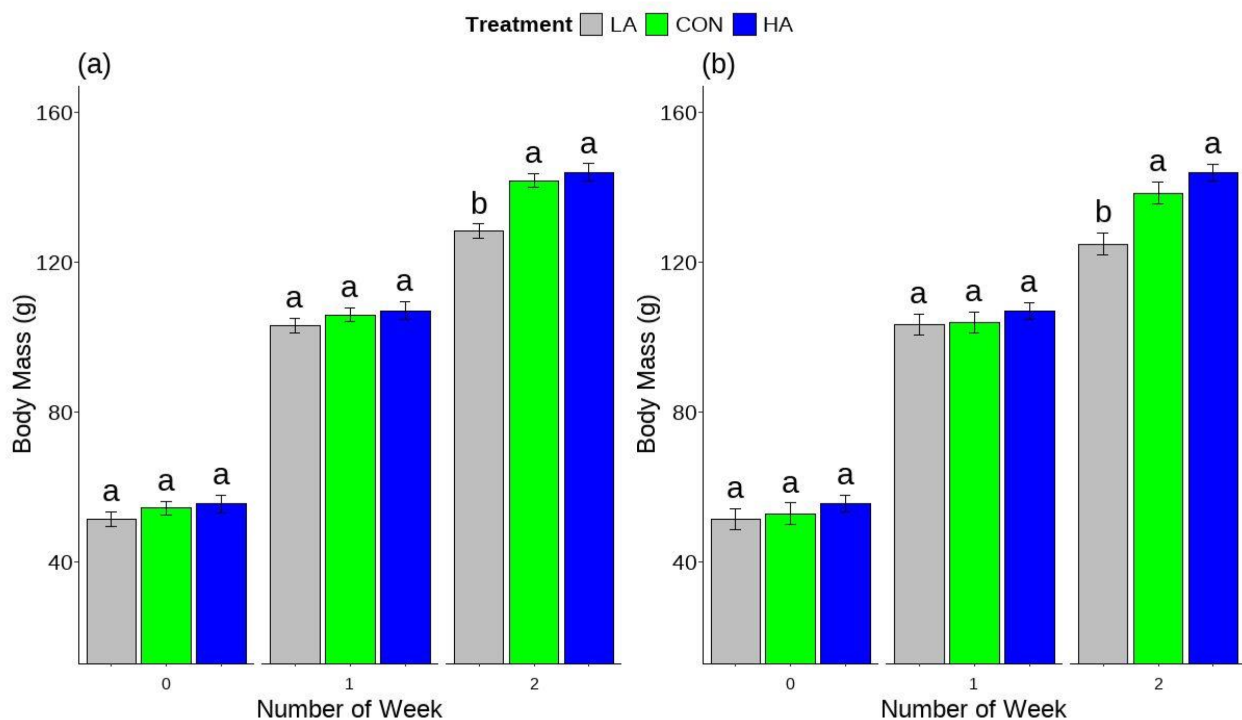


Fig 1. Effect of dietary arginine manipulation on body mass of (a) female and (b) male Japanese quail in a two-week treatment period. The low-arginine groups showed significantly lower body weight compared to the control and high-arginine groups in both sexes at the second week of the experimental period. Data are means ± SEM. Different letters indicate significant differences at a given time point at p ≤ 0.05. LA: low arginine, CON: control, HA: high arginine.

Sex-specific effects of dietary arginine on mTOR pathway gene expression

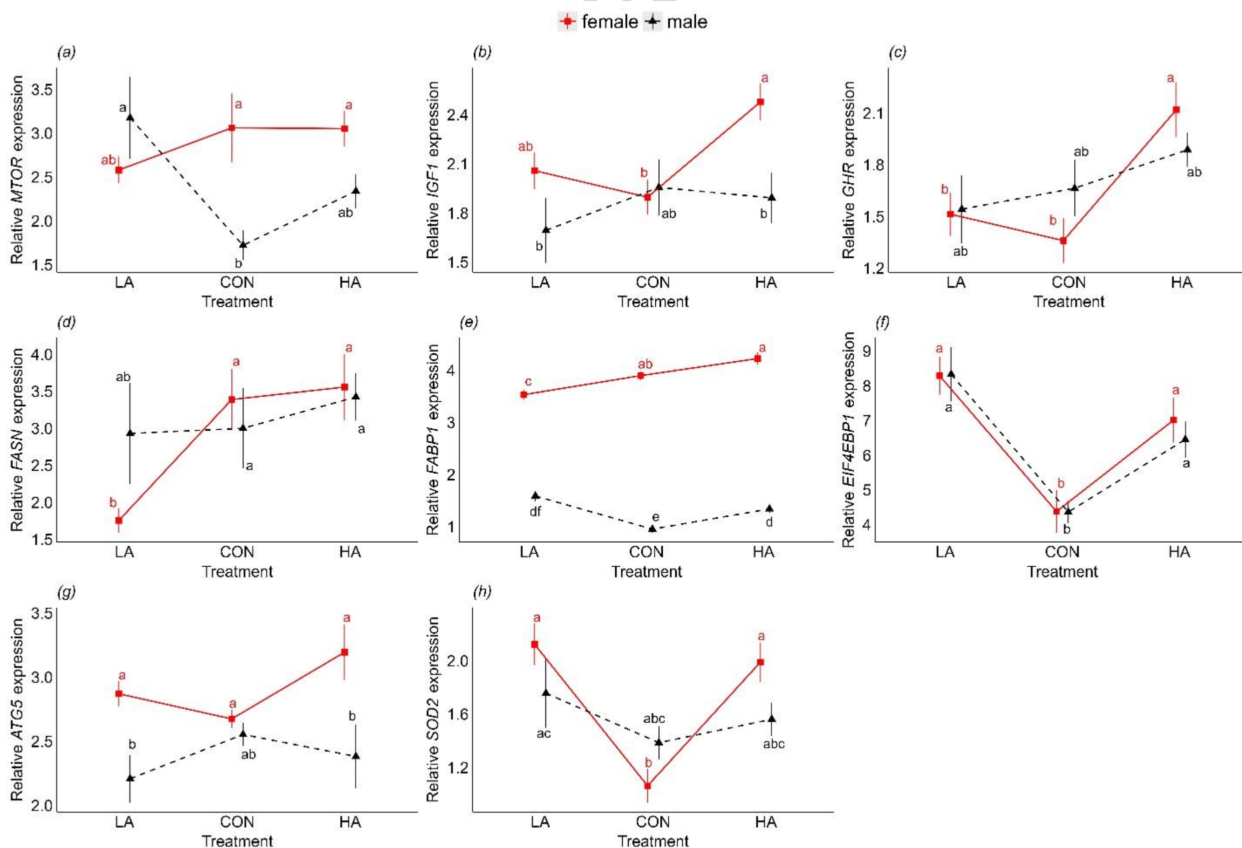
Dietary arginine manipulation significantly altered gene expression in the mTOR signalling pathway, with distinct sex-specific responses (Fig. 2). Arginine restriction significantly

ARTICLE IN PRESS

upregulated the expression of *MTOR* ($p = 0.002$; Fig. 2a) and *FABP1* ($p < 0.001$; Fig. 2e) in males, while *EIF4EBP1* ($p < 0.001$; Fig. 2f) in both sexes. In females, arginine restriction reduced the expression of fatty acid-related *FASN* ($p = 0.032$; Fig. 2d) and *FABP1* ($p < 0.001$; Fig. 2e) while elevating antioxidant *SOD2* ($p < 0.001$; Fig. 2h). On the contrary, arginine supplementation upregulated several targets, including the growth-related genes *IGF1* ($p = 0.016$; Fig. 2b) and *GHR* ($p < 0.001$; Fig. 2c) and also *SOD2* ($p < 0.001$; Fig. 2h) in females, the lipid transport gene *FABP1* ($p = 0.012$; Fig. 2e) in males, and *EIF4EBP1* in both sexes (males: $p = 0.022$; females: $p = 0.016$; Fig. 2f).

Gene expression exhibited notable sex-specific variation across treatments, particularly in genes associated with growth, metabolism, and autophagy (Fig. 2). Arginine restriction in female birds showed higher expression of *FABP1* ($p < 0.001$; Fig. 2e) and *ATG5* ($p = 0.008$; Fig. 2g), while *IGF1* ($p = 0.005$; Fig. 2b), *FABP1* ($p < 0.001$; Fig. 2e), and *ATG5* ($p < 0.001$; Fig. 2h) in the arginine supplementation diet and during control conditions; only *MTOR* and *FABP1* were different (Fig. 2a, e).

ARTICLE IN PRESS



ARTICLE IN PRESS

Fig 2. Sex-specific gene expression. (a) *MTOR*, mechanistic target of rapamycin. (b) *IGF1*, insulin-like growth factor 1; (c) *GHR*, growth hormone receptor; (d) *FASN*, fatty acid synthase; (e) *FABP1*, fatty acid binding protein 1; (f) *EIF4EBP1*, eukaryotic translation initiation factor 4E binding protein 1; (g) *ATG5*, autophagy-related 5; (h) *SOD2*, superoxide dismutase 2. Data are means ± s.e.m from 8 birds per group and were analysed using ANOVA. Different letters indicate significant differences at a given time point at $p \leq 0.05$. LA: low arginine, CON: control, HA: high arginine.

Gene network analysis

Gene co-expression network analysis revealed that dietary arginine availability modulates the overall structure and efficiency of molecular interactions in the liver, with marked sex-specific differences (Fig. 3, Table 3). Arginine supplementation increased gene connectivity and integration, particularly in females, as shown by higher degree and closeness centrality values (Fig. 3c). Key regulatory genes such as *EIF4EBP1* and *ATG5* became more central under high-arginine diets in females, suggesting coordinated upregulation of growth and cellular maintenance pathways. In contrast, males display more variable responses across diets. Notably, *mTOR* and *IGF1* exhibit higher betweenness and centrality in arginine-restricted groups (Fig. 3d), indicating their pivotal role in maintaining network function under nutrient restriction. The control group exhibited more balanced, intermediate overall connectivity in both sexes (Fig. 3b, e), suggesting a baseline level of regulatory coordination that is either enhanced or disrupted by dietary manipulation.

ARTICLE IN PRESS

ARTICLE IN PRESS

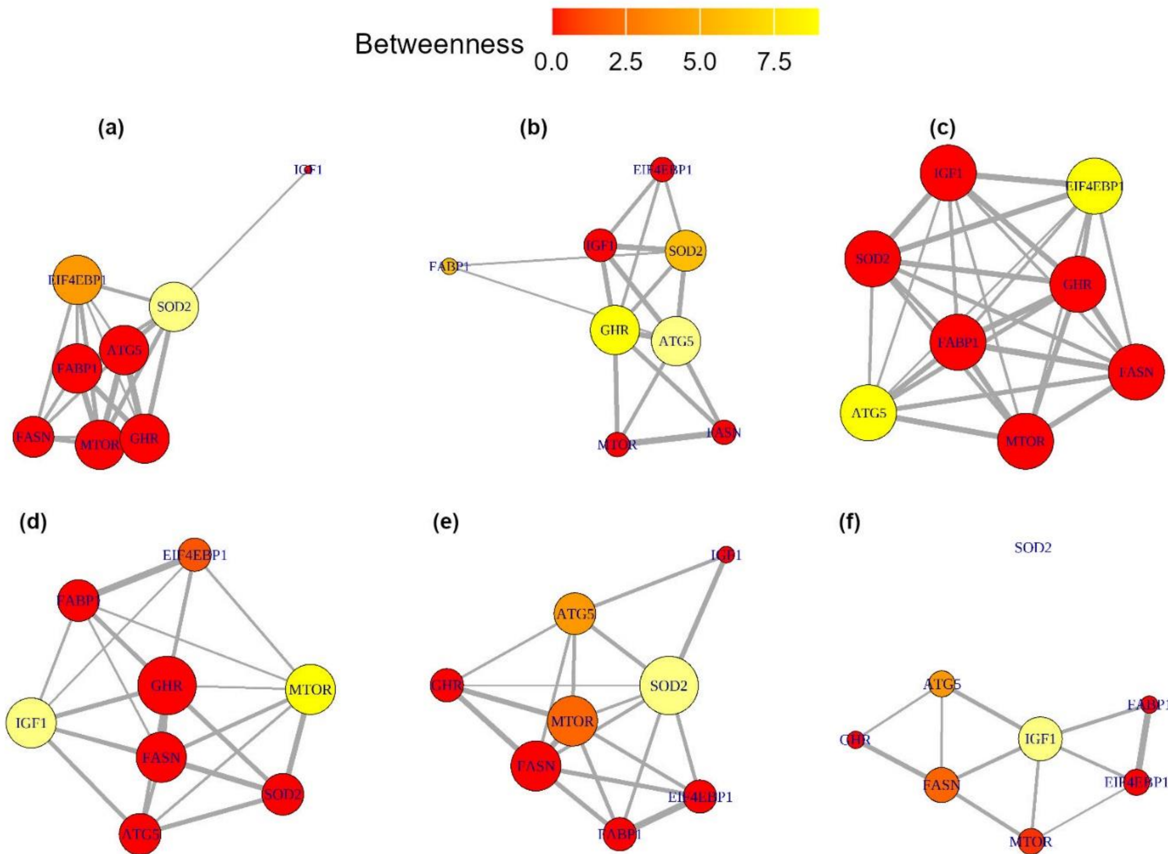


Fig 3. Gene network analysis. (a) female: low arginine treatment, (b) female: control treatment, (c) female: high arginine treatment, (d) male: low arginine treatment, (e) male: control treatment, (f) male: high arginine treatment. *MTOR*, mechanistic target of rapamycin; *IGF1*, insulin-like growth factor 1; *GHR*, growth hormone receptor; *FASN*, fatty acid synthase; *FABP1*, fatty acid binding protein 1; *EIF4EBP1*, eukaryotic translation initiation factor 4E binding Protein 1; *ATG5*, autophagy-related 5; *SOD2*, superoxide dismutase. Each gene is represented as a node, and significant correlations between gene expression values ($r > 0.3$) are represented as edges. The node size (ranges from 0 - 7; where 0 degree is no connectedenss whereas 7 degree is high connectedness) reflects degree of centrality which indicating how many direct connections a gene has, emphasising the importance of connections with high-degree genes (hubs) to the overall network function, while the node colour represents betweenness centrality, with yellow indicating high and red indicating low centrality reflects how often it lies on the shortest paths between other genes. Closeness centrality assesses how close a gene is to all other genes in the network, providing insight into the efficiency of information flow through that gene. The edge thickness corresponds to the strength of the correlation between genes.

ARTICLE IN PRESS

Table 3. The effect of treatments and sex on centrality measures

Gene	Sex	Degree			Betweenness			Closeness		
		LA	CON	HA	LA	CON	HA	LA	CON	HA
MTOR	Female	6	3	7	0	0	0	0.178	0.147	0.207
IGF1	Female	1	4	7	0	0	0	0.148	0.148	0.231
GHR	Female	6	6	7	0	4	0	0.203	0.204	0.177
FASN	Female	5	3	7	0	0	0	0.196	0.143	0.199
FABP1	Female	6	2	7	0	3	0	0.202	0.183	0.194
EIF4EBP1	Female	6	3	7	3	0	0	0.261	0.173	0.233
ATG5	Female	6	6	7	0	5	1	0.194	0.199	0.233
SOD2	Female	6	5	7	6	3	0	0.214	0.185	0.200

ARTICLE IN PRESS

MTOR	Male	6	6	3	3	2	1	0.271	0.207	0.213
IGF1	Male	6	2	5	4	0	6	0.261	0.143	0.272
GHR	Male	7	4	2	0	0	0	0.226	0.203	0.156
FASN	Male	6	6	4	0	0	2	0.209	0.196	0.235
FABP1	Male	5	4	2	0	0	0	0.237	0.165	0.158
EIF4EBP1	Male	4	4	3	1	0	0	0.221	0.164	0.187
ATG5	Male	5	5	3	0	3	3	0.226	0.208	0.213
SOD2	Male	5	7	0	0	6	0	0.199	0.267	Na

LA low arginine, CON control, HA high arginine, Na, not applicable

ARTICLE IN PRESS

Discussion

The study demonstrated that early post-hatch dietary arginine availability affects growth and gene expression in Japanese quail. Arginine restriction led to growth retardation in both sexes, indicating that adequate dietary arginine is essential for normal development [50,51]. However, gene expression responses showed clear sex-specific patterns. In the present study, although arginine supplementation did not enhance growth significantly, it triggered changes in gene expression,

ARTICLE IN PRESS

particularly related to growth regulation and oxidative stress. By separating the effects of arginine restriction and supplementation, we clarified how changes in dietary arginine influence growth-related molecular pathways. These findings indicate that grower quails are sensitive to early nutritional conditions and suggest differences in metabolic regulation between the sexes, even without visible differences in body mass.

The somatotropic GHR-IGF1-mTOR axis tightly regulates avian growth, with IGF1 serving as a reliable marker of growth potential due to its role in promoting protein synthesis and muscle development [52,53]. In our study, arginine supplementation increased hepatic *IGF1* and *GHR* in females (Fig. 2b, c), while males showed no significant changes, which aligns with the notion that male hepatic *IGF1* and *GHR* expression are less responsive to environmental factors[54]. Despite elevated *IGF1* and *GHR* in females, body mass did not differ significantly between sexes under arginine supplementation conditions, suggesting a potential lag between transcriptional activation and physical growth [28]. Under arginine restriction, *IGF1* and *GHR* expression remained unchanged in both sexes, consistent with the observed growth retardation. These results indicate that arginine supplementation enhances *IGF1* and *GHR* expression primarily in females, potentially amplifying growth signalling, while arginine deficiency suppresses growth without altering gene expression [28,55,56]. The sex-specific *IGF1* response highlights females exhibiting greater molecular sensitivity to dietary arginine, which could support growth under nutrient-rich conditions[57].

ARTICLE IN PRESS

As a downstream effector of IGF1, mTOR integrates arginine availability to regulate protein synthesis and growth, linking dietary inputs to the GHR-IGF1 axis. Arginine activates mTOR through several mechanisms [4,58–60]. In our study, male quails in the arginine restriction group showed significantly increased *MTOR* expression, potentially compensating for limited arginine to sustain protein synthesis, though insufficient to prevent growth retardation. Because IGF1 levels in males remained stable across diets, the rise in *MTOR* under arginine restriction likely reflects an IGF1-independent compensatory response to low intracellular arginine. However, females displayed higher basal *MTOR* expression under the control diet than males (Fig. 2a), suggesting a sex-specific regulatory pattern which might be hormonal factors [19,61–636061]. Thus, females may maintain a stable mTOR activity through hormonal modulation, regardless of dietary differences. indicate that arginine modulates the GHR-IGF1-mTOR axis differently by sex.

ARTICLE IN PRESS

Fatty acid metabolism, crucial for energy storage and reproductive preparation, is regulated by arginine through mTOR, influencing lipogenic gene expression [64]. In our study, the expression of *FASN,* a central lipogenetic enzyme, was downregulated in females under the arginine-restricted diet, while males showed no significant differences across treatments (Fig. 2d). This suggests that arginine deficiency impairs lipid synthesis more prominently in females, potentially exacerbating growth retardation by limiting energy reserves and reflecting sex-related metabolic differences. To complement this, we also analysed *FABP1*, which facilitates intracellular fatty acid transport and metabolism. *FABP1* was expressed at higher levels in females than males across all treatments (Fig. 2e). However, specifically, males in both arginine-restricted and supplemented groups showed moderately elevated expression compared to the control diet group. This consistent sex-specific expression may reflect differences in lipid metabolic regulation, potentially influenced by early hormonal activity, as females exhibit higher lipid mobilisation and storage capacity [65–68]. These sex-specific patterns indicate that females prioritise lipid transport and storage, potentially driven by IGF1 and mTOR signalling under arginine-supplemented conditions, supporting growth despite stable *mTOR* expression. In males, *FABP1* upregulation aligns with mTOR activation under arginine restriction, reflecting a compensatory mechanism to maintain lipid metabolism during growth limitation [20].

Downstream of *mTOR*, *EIF4EBP1* regulates translation and metabolic processes critical for growth and energy homeostasis, further mediating arginine's effects on the GHR-IGF1-mTOR axis [69]. In our study, *EIF4EBP1* expression was significantly upregulated in both sexes under arginine restriction and supplementation (Fig. 2f). Unlike *IGF1*, *mTOR*, and *FABP1*, *EIF4EBP1* showed no sex-specific differences, suggesting a conserved response to arginine availability. Under arginine restriction, *EIF4EBP1* upregulation in both sexes likely inhibits translation initiation, contributing to growth retardation by limiting protein synthesis, consistent with reduced body mass. In contrast, under high-arginine diets, *EIF4EBP1* upregulation may fine-tune translation to support metabolic adaptation, particularly in females. This bidirectional 4EBP1 response aligns with mTOR activation in males under low arginine and IGF1-driven signalling in females under high arginine, reinforcing arginine's role in modulating growth through the GHR-IGF1-mTOR axis [70], which indicates that 4EBP1 acts as a key regulator of arginine-mediated metabolic responses.

ARTICLE IN PRESS

In addition to translation regulation, mTOR modulates autophagy via ATG5, an essential gene that recycles cellular components during nutrient stress, influencing growth and metabolic adaptation [71,72]. In our study, female quails exhibited significantly higher *ATG5* expression than males under both arginine-restricted and supplemented groups, indicating a sex-specific response to arginine availability. In females, *ATG5* upregulation under arginine restriction likely enhances cellular recycling to mitigate nutrient stress, contributing to growth retardation alongside *EIF4EBP1* and *FASN* responses. Under arginine-supplemented diets, female *ATG5* upregulation may support metabolic flexibility, complementing *IGF1* and *FABP1* to sustain growth potential [27]. The inverse relationship between mTOR activation and autophagy [73] explains these patterns: male *mTOR* upregulation under low arginine suppresses *ATG5*, while female IGF1-driven mTOR signalling under high arginine allows *ATG5* expression to persist.

Beyond autophagy, mTOR regulates oxidative stress responses via SOD2, a mitochondrial antioxidant enzyme that protects cells from reactive oxygen species during growth [12]. In our study, females exhibited significant *SOD2* upregulation under restricted and supplemented diets (Fig. 2i), while males showed no treatment effects. Despite this female-specific response, overall sex differences were not statistically significant, indicating a conserved role for *SOD2* in both sexes. In females, elevated *SOD2* under arginine restriction likely enhances antioxidant defence during growth retardation, complementing *ATG5* and *EIF4EBP1*. Under high-arginine diets, *SOD2* upregulation complements *IGF1*, *FABP1*, and *ATG5*, potentially protecting against reactive oxygen species to sustain growth potential. These suggest that arginine modulates *SOD2* expression via the GHR-IGF1-mTOR axis, with females showing greater sensitivity that may contribute to metabolic resilience [35,74].

In this study, gene network analysis further elucidates these sex-specific responses, with betweenness centrality highlighting key hubs. Under arginine restriction, the female network (Fig. 3a) showed high *SOD2* and *EIF4EBP1* betweenness, reflecting their roles in antioxidant defence and translation regulation despite growth challenges, while the male network (Fig. 3d) was sparse with *mTOR* and *IGF1* as central hubs, aligning with a focus on protein synthesis and growth signalling. Under arginine supplementation, the female network (Fig. 3c) exhibited high *EIF4EBP1* and *ATG5* betweenness, consistent with enhanced translation and autophagy to support metabolic adaptation, whereas the male network (Fig. 3f) emphasised *IGF1*, indicating limited responsiveness in growth signalling. The denser female networks across treatments underscore

ARTICLE IN PRESS

ARTICLE IN PRESS

their greater molecular integration, supporting the hypothesis that arginine modulates the GHR-IGF1-mTOR axis to drive sex-specific growth and metabolic adaptations. These patterns are influenced by sex and nutrient availability [6,27]. However, gene network connectivity in poultry is highly complex, involving multiple interacting genes that regulate key traits [75,76].

In conclusion, this study reveals the critical role of dietary arginine in shaping growth, acting through the GHR-IGF1-mTOR signalling axis. Arginine limitation impairs body mass across sexes, disrupts network connectivity, particularly in males, where sparse networks with *MTOR* and *IGF1* hubs suppress growth potential. In contrast, in females, arginine supplementation induced changes in gene expression, which refers to molecular-level regulation rather than phenotypic gain, including the upregulation of growth-related (*IGF1*, *GHR)*, translation (*EIF4EBP1*), autophagy (*ATG5*), and oxidative defence (*SOD2*) genes. These molecular shifts, together with denser female networks centred on *SOD2* and *ATG5*, suggest greater female resilience in integrating nutritional signals. Consistent *EIF4EBP1* upregulation across sexes highlights its central role in linking arginine availability to metabolic regulation. Overall, these findings provide new insight into how early nutritional conditions shape sex-specific metabolic response trajectories in birds. Examining the GHR–IGF1–mTOR axis in additional tissues and over extended periods could shed light on arginine's variable effects and guide refined nutritional strategies.

ARTICLE IN PRESS

Data availability

All data generated or analysed during this study are included in this published article and its supplementary information files.

REFERENCES

1 Simpson SJ, Raubenheimer D. *The Nature of Nutrition*. Princeton University Press, 2012 doi:10.23943/princeton/9780691145655.001.0001.

2 Buchanan KL, Meillère A, Jessop TS. Early Life Nutrition and the Programming of the Phenotype. Development Strategies and Biodiversity, 2022, pp 161–214.

3 Prabakar G L, A, Kumaravel P, Chahande JM, Majumder J, Gangopadhyay S, Biswas S *et al.* Advancements in Animal Nutrition an Insights from Veterinary Science. *Uttar pradesh journal of zoology* 2024; **45**: 139–145.

4 Carroll B, Maetzel D, Maddocks OD, Otten G, Ratcliff M, Smith GR *et al.* Control of TSC2-Rheb signaling axis by arginine regulates mTORC1 activity. *Elife* 2016; **5**. doi:https://doi.org/10.7554/eLife.11058.

ARTICLE IN PRESS

5 Takahara T, Amemiya Y, Sugiyama R, Maki M, Shibata H. Amino acid-dependent control of mTORC1 signaling: a variety of regulatory modes. *J Biomed Sci* 2020; **27**: 87.

6 Ndunguru SF, Reda GK, Csernus B, Knop R, Lugata JK, Szabó C *et al.* Embryonic Leucine Promotes Early Postnatal Growth via mTOR Signalling in Japanese Quails. *Animals* 2024; **14**: 2596.

7 Laplante M, Sabatini DM. MTOR signaling in growth control and disease. Cell. 2012; **149**: 274–293.

8 Regan JC, Froy H, Walling CA, Moatt JP, Nussey DH. Dietary restriction and insulin-like signalling pathways as adaptive plasticity: A synthesis and re-evaluation. *Funct Ecol* 2020; **34**: 107–128.

9 Abdullah HafizM, Bielke LR, Helmy YA. EFFECT OF ARGININE SUPPLEMENTATION ON GROWTH PERFORMANCE AND IMMUNITY OF BROILERS: A REVIEW. *Journal of Global Innovations in Agricultural and Social Sciences* 2019; **7**: 141–144.

10 Khajali F, Wideman RF. Dietary arginine: metabolic, environmental, immunological and physiological interrelationships. *Worlds Poult Sci J* 2010; **66**: 751–766.

11 Wu G. Amino acids: Metabolism, functions, and nutrition. Amino Acids. 2009; **37**: 1–17.

12 Atakisi O, Atakisi E, Kart A. Effects of Dietary Zinc and l-Arginine Supplementation on Total Antioxidants Capacity, Lipid Peroxidation, Nitric Oxide, Egg Weight, and Blood Biochemical Values in Japanase Quails. *Biol Trace Elem Res* 2009; **132**: 136–143.

13 Gao T, Zhao M, Zhang L, Li J, Yu L, Gao F *et al. In ovo* feeding of <scp>l</scp> - arginine regulates intestinal barrier functions of posthatch broilers by activating the mTOR signaling pathway. *J Sci Food Agric* 2018; **98**: 1416–1425.

14 Zhou M, Wei Y, Feng Y, Zhang S, Ma N, Wang K *et al.* Arginine Regulates Skeletal Muscle Fiber Type Formation via mTOR Signaling Pathway. *Int J Mol Sci* 2024; **25**: 6184.

15 Kim E, Goraksha-Hicks P, Li L, Neufeld TP, Guan KL. Regulation of TORC1 by Rag GTPases in nutrient response. *Nat Cell Biol* 2008; **10**: 935–945.

16 Wang X, Zhang T, Li W, Wang H, Yan L, Zhang X *et al.* Arginine alleviates Clostridium perfringens α toxin-induced intestinal injury in vivo and in vitro via the SLC38A9/mTORC1 pathway. *Front Immunol* 2024; **15**. doi:10.3389/fimmu.2024.1357072.

17 McDonald JMC, Nabili P, Thorsen L, Jeon S, Shingleton AW. Sex-specific plasticity and the nutritional geometry of insulin-signaling gene expression in Drosophila melanogaster. *Evodevo* 2021; **12**: 6.

ARTICLE IN PRESS

ARTICLE IN PRESS

18 Bonneaud C, Marnocha E, Herrel A, Vanhooydonck B, Irschick DJ, Smith TB. Developmental plasticity affects sexual size dimorphism in an anole lizard. *Funct Ecol* 2016; **30**: 235–243.

19 Gürgen D, Kusch A, Klewitz R, Hoff U, Catar R, Hegner B *et al.* Sex-Specific mTOR Signaling Determines Sexual Dimorphism in Myocardial Adaptation in Normotensive DOCA-Salt Model. *Hypertension* 2013; **61**: 730–736.

20 Reda GK, Ndunguru SF, Csernus B, Lugata JK, Knop R, Szabó C *et al.* Sex-specific effects of dietary restriction on physiological variables in Japanese quails. *Ecol Evol* 2024; **14**. doi:10.1002/ece3.11405.

21 Sun S-X, Wu J-L, Lv H-B, Zhang H-Y, Zhang J, Limbu SM *et al.* Environmental estrogen exposure converts lipid metabolism in male fish to a female pattern mediated by AMPK and mTOR signaling pathways. *J Hazard Mater* 2020; **394**: 122537.

22 Caetano-Anolles K, Seo M, Rodriguez-Zas S, Oh J-D, Han JY, Lee K *et al.* Comprehensive Identification of Sexual Dimorphism-Associated Differentially Expressed Genes in Two-Way Factorial Designed RNA-Seq Data on Japanese Quail (Coturnix coturnix japonica). *PLoS One* 2015; **10**: e0139324.

23 Harper JM, Holmes DJ. New Perspectives on Avian Models for Studies of Basic Aging Processes. *Biomedicines* 2021; **9**: 649.

24 Minvielle F. The future of Japanese quail for research and production. In: *World's Poultry Science Journal*. 2004, pp 500–507.

25 Randall M, Bolla G. J A N U A R Y 2 0 0 8 Raising Japanese quail. 2008.

26 Yu LL, Gao T, Zhao MM, Lv PA, Zhang L, Li JL *et al.* Effects of in ovo feeding of l-arginine on breast muscle growth and protein deposition in post-hatch broilers. *Animal* 2018; **12**: 2256–2263.

27 Reda GK, Ndunguru SF, Csernus B, Gulyás G, Knop R, Szabó C *et al.* Dietary restriction and life-history trade-offs: insights into mTOR pathway regulation and reproductive investment in Japanese quail. *Journal of Experimental Biology* 2024; **227**. doi:10.1242/jeb.247064.

28 Ndunguru SF, Reda GK, Csernus B, Knop R, Gulyás G, Szabó C *et al.* Embryonic methionine triggers post-natal developmental programming in Japanese quail. *Journal of Comparative Physiology B* 2024; **194**: 179–189.

29 Reda GK, Ndunguru SF, Csernus B, Knop R, Lugata JK, Szabó C *et al.* Dietary restriction reveals sex-specific expression of the mTOR pathway genes in Japanese quails. *Sci Rep* 2024; **14**: 8314.

30 Cui HX, Zheng MQ, Liu RR, Zhao GP, Chen JL, Wen J. Liver dominant expression of fatty acid synthase (FAS) gene in two chicken breeds during intramuscular-fat development. *Mol Biol Rep* 2012; **39**: 3479–3484.

ARTICLE IN PRESS

ARTICLE IN PRESS

31 Fouad AM, El-Senousey HK, Yang XJ, Yao JH. Dietary L-arginine supplementation reduces abdominal fat content by modulating lipid metabolism in broiler chickens. *Animal* 2013; **7**: 1239–1245.

32 Wang Z, Yue Y-X, Liu Z-M, Yang L-Y, Li H, Li Z-J *et al.* Genome-Wide Analysis of the FABP Gene Family in Liver of Chicken (Gallus gallus): Identification, Dynamic Expression Profile, and Regulatory Mechanism. *Int J Mol Sci* 2019; **20**: 5948.

33 Aryal B, Kwakye J, Ariyo OW, Ghareeb AFA, Milfort MC, Fuller AL *et al.* Major Oxidative and Antioxidant Mechanisms During Heat Stress-Induced Oxidative Stress in Chickens. *Antioxidants* 2025; **14**: 471.

34 DeMoranville KJ, Carter WA, Pierce BJ, McWilliams SR. Flight and Dietary Antioxidants Influence Antioxidant Expression and Activity in a Migratory Bird. *Integrative Organismal Biology* 2022; **4**. doi:10.1093/iob/obab035.

35 Surai F P. Antioxidant Systems in Poultry Biology: Superoxide Dismutase. *Journal of Animal Research and Nutrition* 2016; **01**. doi:10.21767/2572-5459.100008.

36 AHMAD F, SHARIF M, ASHRAF M, RIAZ M, SHOAIB M, SIDDIQUE T *et al.* Effect of different light intensities and colors on growth performance and reproductive characteristics in Japanese quails. *Turk J Vet Anim Sci* 2023; **47**: 185–193.

37 National Research Council (U.S.). Subcommittee on Poultry Nutrition. *Nutrient requirements of poultry*. National Academy Press, 1994.

38 Fathima S, Hakeem WG Al, Shanmugasundaram R, Selvaraj RK. Effect of arginine supplementation on the growth performance, intestinal health, and immune responses of broilers during necrotic enteritis challenge. *Poult Sci* 2024; **103**: 103815.

39 Fathima S, Al Hakeem WG, Shanmugasundaram R, Periyannan V, Varadhan R, Selvaraj RK. Effect of 125% and 135% arginine on the growth performance, intestinal health, and immune responses of broilers during necrotic enteritis challenge. *Poult Sci* 2024; **103**: 103826.

40 Maurício TV, Vargas Júnior JG de, Souza MF de, Barboza WA, Nunes LDC, Soares RTRN *et al.* Digestible arginine concentrations in the diet of Japanese quails. *Semin Cienc Agrar* 2016; **37**: 2453.

41 Simon, Jávor A, Bai P, Oláh J, Czeglédi L. Reference gene selection for reverse transcription quantitative polymerase chain reaction in chicken hypothalamus under different feeding status. *J Anim Physiol Anim Nutr (Berl)* 2018; **102**: 286–296.

42 Joshi CJ, Ke W, Drangowska-Way A, O'Rourke EJ, Lewis NE. What are housekeeping genes? *PLoS Comput Biol* 2022; **18**. doi:10.1371/journal.pcbi.1010295.

43 Livak KJ, Schmittgen TD. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. *Methods* 2001; **25**: 402–408.

ARTICLE IN PRESS

ARTICLE IN PRESS

44 R Core Team. R: A Language and Environment for Statistical Computing. 2024 doi:http://www.R-project.org/.

45 Bates D, Mächler M, Bolker BM, Walker SC. Fitting linear mixed-effects models using lme4. *J Stat Softw* 2015; **67**. doi:10.18637/jss.v067.i01.

46 Vaida F, Blanchard S. Conditional Akaike information for mixed-effects models. 2005https://academic.oup.com/biomet/article/92/2/351/233128.

47 Kuznetsova A, Brockhoff PB, Christensen RHB. lmerTest Package: Tests in Linear Mixed Effects Models. *J Stat Softw* 2017; **82**: 1–26.

48 Searle SR, Speed FM, Milliken GA. Population marginal means in the linear model: An alternative to least squares means. *American Statistician* 1980; **34**: 216–221.

49 Zhang B, Horvath S. A General Framework for Weighted Gene Co-Expression Network Analysis. *Stat Appl Genet Mol Biol* 2005; **4**. doi:10.2202/1544-6115.1128.

50 Al-Daraji HJ, Salih AM. Effect of Dietary L-Arginine on Productive Performance of Broiler Chickens. *Pakistan Journal of Nutrition* 2012; **11**: 252–257.

51 Sousa M, Lima M, Vieira RB, Pavanini J, Peruzzi NJ, Raimundo E *et al.* Modeling the response of Japanese quail to arginine intake. *PeerJ* 2022; **10**: e14337.

52 Burnside J, Cogburn LA. Developmental expression of hepatic growth hormone receptor and insulin-like growth factor-I mRNA in the chicken. *Mol Cell Endocrinol* 1992; **89**: 91–96.

53 Beccavin C, Chevalier B, Cogburn L, Simon J, Duclos M. Insulin-like growth factors and body growth in chickens divergently selected for high or low growth rate. *Journal of Endocrinology* 2001; **168**: 297–306.

54 Sur S, Stewart C, Liddle TA, Monteiro AM, Denizli I, Majumdar G *et al.* Molecular basis of photoinduced seasonal energy rheostasis in Japanese quail (Coturnix japonica). *Mol Cell Endocrinol* 2025; **595**: 112415.

55 Harvey S. Growth hormone and growth? *Gen Comp Endocrinol* 2013; **190**: 3–9.

56 Scanes CG. Perspectives on the endocrinology of poultry growth and metabolism. *Gen Comp Endocrinol* 2009; **163**: 24–32.

57 Saxena R, Saxena VK, Tripathi V, Mir NA, Dev K, Begum J *et al.* Dynamics of gene expression of hormones involved in the growth of broiler chickens in response to the dietary protein and energy changes. *Gen Comp Endocrinol* 2020; **288**: 113377.

58 Saxton RA, Chantranupong L, Knockenhauer KE, Schwartz TU, Sabatini DM. Mechanism of arginine sensing by CASTOR1 upstream of mTORC1. *Nature* 2016; **536**: 229–233.

ARTICLE IN PRESS

59 Jung JW, Macalino SJY, Cui M, Kim JE, Kim H-J, Song D-G *et al.* Transmembrane 4 L Six Family Member 5 Senses Arginine for mTORC1 Signaling. *Cell Metab* 2019; **29**: 1306-1319.e7.

60 Meng D, Yang Q, Wang H, Melick CH, Navlani R, Frank AR *et al.* Glutamine and asparagine activate mTORC1 independently of Rag GTPases. *Journal of Biological Chemistry* 2020; **295**: 2890–2899.

61 Yao K, Yin Y-L, Chu W, Liu Z, Deng D, Li T *et al.* Dietary Arginine Supplementation Increases mTOR Signaling Activity in Skeletal Muscle of Neonatal Pigs ,. *J Nutr* 2008; **138**: 867–872.

62 Udoh UH, Udoh JE, Adeoye AA. Evaluation of Sex Effects on Serum Biochemical and Genetic Parameters of Japanese Quails. *Asian Research Journal of Agriculture* 2021; : 113–122.

63 Hao E, Liu X, Chang L, Xue H, Su B, Chen Y *et al.* Melatonin alleviates endoplasmic reticulum stress to improve ovarian function by regulating the mTOR pathway in aged laying hens. *Poult Sci* 2024; **103**: 103703.

64 Saxton RA, Sabatini DM. mTOR Signaling in Growth, Metabolism, and Disease. *Cell* 2017; **168**: 960–976.

65 Ding L, Chen J, He F, Chen Q, Li Y, Chen W. Effects of dietary arginine supplementation on growth performance, antioxidant capacity, intestinal digestive enzyme activity, muscle transcriptome, and gut health of Siniperca chuatsi. *Front Mar Sci* 2024; **10**. doi:10.3389/fmars.2023.1305192.

66 Harper JM, Wang M, Galecki AT, Ro J, Williams JB, Miller RA. Fibroblasts from long-lived bird species are resistant to multiple forms of stress. *Journal of Experimental Biology* 2011; **214**: 1902–1910.

67 Haunerland NH, Spener F. Fatty acid-binding proteins – insights from genetic manipulations. *Prog Lipid Res* 2004; **43**: 328–349.

68 Hermier D. Lipoprotein Metabolism and Fattening in Poultry. *J Nutr* 1997; **127**: 805S-808S.

69 Cho S-Y, Lee S, Yeom J, Kim H-J, Lee J-H, Shin J-W *et al.* Transglutaminase 2 mediates hypoxia-induced selective mRNA translation via polyamination of 4EBPs. *Life Sci Alliance* 2020; **3**: e201900565.

70 Ryoo HD. The integrated stress response in metabolic adaptation. *Journal of Biological Chemistry* 2024; **300**: 107151.

71 Meijer AJ, Lorin S, Blommaart EF, Codogno P. Regulation of autophagy by amino acids and MTOR-dependent signal transduction. *Amino Acids* 2015; **47**: 2037–2063.

72 Meijer AJ, Codogno P. Regulation and role of autophagy in mammalian cells. *Int J Biochem Cell Biol* 2004; **36**: 2445–2462.

ARTICLE IN PRESS

ARTICLE IN PRESS

73 Ye X, Zhou X-J, Zhang H. Exploring the Role of Autophagy-Related Gene 5 (ATG5) Yields Important Insights Into Autophagy in Autoimmune/Autoinflammatory Diseases. *Front Immunol* 2018; **9**. doi:10.3389/fimmu.2018.02334.

74 Oke OE, Akosile OA, Oni AI, Opowoye IO, Ishola CA, Adebiyi JO *et al.* Oxidative stress in poultry production. *Poult Sci* 2024; **103**: 104003.

75 Jaffrezic F, Tosser-Klopp G. Gene network reconstruction from microarray data. *BMC Proc* 2009; **3**: S12.

76 Stanley D, Watson-Haigh NS, Cowled CJ, Moore RJ. Genetic architecture of gene expression in the chicken. *BMC Genomics* 2013; **14**: 13.

ACKNOWLEDGMENTS

We thank Aya Ferroudj and Joab Osti Malanda for their help during the experiment. We acknowledge the Molecular lab of the Animal Science Department at the University of Debrecen for providing the necessary lab facilities. We also thank the members of the eco-physiology lab of the Department of Evolutionary Zoology and Human Biology for their discussions and insightful inputs into the manuscript. This study is part of the doctoral dissertation of the first author.

ARTICLE IN PRESS

AUTHOR CONTRIBUTIONS

Conceptualisation: M.G, G.K.R., Á.Z.L.; L.C., Methodology: M.G., G.K.R., B.C., G.G., R.K., C.S., Á.Z.L.; L.C., Software: M.G., G.K.R., Á.Z.L.; Validation: M.G., Á.Z.L.; L.C., Formal analysis: M.G.; Investigation: M.G., Resources: M.G., Á.Z.L.; L.C., Data curation: M.G.; Writing - original draft: M.G.; Writing - review & editing: M.G., G.K.R., F.N.A., E. M.A. D.M.K., S.F.N., B.C., G.G., R.K., C.S., Á.Z.L.; L.C., Visualization: M.G., G.K.R.,Á.Z.L.; Supervision: Á.Z.L.; L.C., Project administration: Á.Z.L.; L.C., Funding acquisition: Á.Z.L., L.C.

FUNDING

The study was supported by the National Development, Research and Innovation Office, Hungary (K139021). M.G., F.N.A., E.M.A., and D.M.K. are supported by a Stipendium Hungaricum Scholarship from Tempus Public Foundation for PhD studies.

COMPETING INTERESTS

The authors declare no competing or financial interests.