The in ovo injection of methionine improves intestinal cell proliferation and differentiation in chick embryos by activating the JAK2/STAT3 signaling pathway

Meng-Jie Chen, Jia-Yi Zhou, Yi-Jun Chen, Xiu-Qi Wang, Hui-Chao Yan, Chun-Qi Gao*

College of Animal Science, South China Agricultural University/Guangdong Provincial Key Laboratory of Animal Nutrition Control, Guangzhou, 510642, China

Original Research Article

The in ovo injection of methionine improves intestinal cell proliferation and differentiation in chick embryos by activating the JAK2/STAT3 signaling pathway

Meng-Jie Chen, Jia-Yi Zhou, Yi-Jun Chen, Xiu-Qi Wang, Hui-Chao Yan, Chun-Qi Gao*

College of Animal Science, South China Agricultural University/Guangdong Provincial Key Laboratory of Animal Nutrition Control, Guangzhou, 510642, China

A R T I C L E   I N F O

Article info
Received 18 October 2020
Received in revised form 25 January 2021
Accepted 9 March 2021
Available online 31 July 2021

Keywords:
In ovo injection
Chick
Intestine
L-methionine
JAK2/STAT3

A B S T R A C T

The intestinal health of chick embryos is vital for their life-long growth, and exogenous nutrition intervention may provide sufficient nutrition for embryonic development. In the present study, we investigated the effect of in ovo injection of L-methionine (L-Met) on the intestinal structure and barrier function of chick embryos. There were 4 groups of treatments: the control (CON) group injected with phosphate-buffered saline (PBS) and the other 3 groups injected with 5, 10, and 20 mg L-Met/egg, respectively. The injection was performed on embryonic day 9 (E9), and intestinal samples were collected on the day of hatching for analysis. The results showed that, compared with the CON group, the groups administered an in ovo injection of L-Met increased relative weights of the duodenum, jejunum, and ileum (P < 0.05). Hematoxylin and eosin (H&E) staining showed that the groups injected with 5, 10, and 20 mg L-Met significantly increased villus height and crypt depth (P < 0.05). Moreover, in ovo injection of 10 mg L-Met also increased the transepithelial electrical resistance (TEER) of the jejunum (P < 0.05). Injection with 10 and 20 mg L-Met increased the expression of the tight junction proteins (ZO-1 and claudin-1) and the fluorescence signal intensity of Ki67 and villin proteins (P < 0.05). Further, the protein expression of phospho-Janus kinase 2 (p-JAK2) and phospho-signal transducer and activator of transcription 3 (p-STAT3) was significantly increased by 10 or 20 mg L-Met injection (P < 0.05). In conclusion, the injection of L-Met, especially at a dose of 10 mg, showed beneficial effects on the intestinal integrity of chick embryos due to the activation of the JAK2/STAT3 signaling pathway. Our results may provide new insights for regulating the intestinal development of embryonic chicks and the rapid growth of chicks after hatching.

© 2021 Chinese Association of Animal Science and Veterinary Medicine. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

The small intestinal epithelium is the major site of nutrient digestion and absorption, and it also provides a natural defense for chicks against pathogens (Peterson and Artis, 2014; Gilbert et al., 2007). To our knowledge, the chick intestine is visible at approximately embryonic day 5 (E5) and then develops continuously to form a typical structure with a crypt–villus axis until the end of hatching (Southwell, 2006). It is noteworthy that the gut grows rapidly in the late hatching period with the marked consumption of egg nutrients, which limits intestinal development and embryo growth (Southwell, 2006; Li et al., 2008; Moran, 2007). Our previous study confirmed that the yolk of chick embryos was completely enclosed by the yolk membrane at E9, which provided reliable time points for nutrition intervention in embryo eggs (Chen et al., 2020a). Therefore, supplementation with exogenous nutrients into eggs may be beneficial for the intestinal development of embryonic chicks and for the rapid growth of chicks after hatching (Willemsen et al., 2010; Kadam et al., 2013).

* Corresponding author.
E-mail address: cqgao@scau.edu.cn (C.-Q. Gao).
Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.

https://doi.org/10.1016/j.aninu.2021.03.009
2405-6545/© 2021 Chinese Association of Animal Science and Veterinary Medicine. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Dietary amino acids are crucial for the gut to potentiate its functions and prevent diseases (Wang et al., 2009). As the first-limiting amino acid in diets for poultry, methionine (Met) has been demonstrated to increase chick weight (Vieira et al., 2004). Moreover, Met is a key regulator of embryonic stem cell differentiation (Shiraki et al., 2014). A previous report involving essential amino acids in enteral nutrition in mice identified that Met deprivation affects the proliferation and differentiation of intestinal stem cells (ISC), which are located at the bottom of the crypts and drive intestinal epithelial renewal and regeneration (Saito et al., 2017). Consistently, supplementation with both Met and its hydroxyl analogs improves intestinal epithelial integrity by promoting the rapid growth of mouse ISC in the context of deoxynivalenol stimulation (Zhou et al., 2019). However, it remains to be further investigated whether the in ovo administration of Met contributes to the intestinal development of chick embryos under homeostasis.

The Janus kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) pathway play a pivotal role in the homeostasis and regeneration of intestinal epithelium (Jiang et al., 2009). Mechanistically, STAT3 is recruited to the activated cytokine receptor and then tyrosine-phosphorylated by receptor-associated JAK2 (Tang et al., 2018). Several studies have confirmed STAT3 as a transcription factor that induces the expression of specific genes, such as cyclin D1, and that is also involved in the transcription of tight junction gene, which regulates the processes of enterocyte proliferation and barrier construction respectively (Yu et al., 2009; Morikawa et al., 2011). Furthermore, STAT3 activation protects the intestinal integrity of mice by accelerating the regeneration of ISC (Hou et al., 2018). Interestingly, Obata et al. (2018) found that S-adenosylmethionine (SAM), the first metabolite of Met, induced ISC division via JAK/STAT. Nevertheless, the mechanism by which Met maintains the integrity of the chick intestinal epithelium, especially the regulation of intestinal cell activity, is still unclear.

Here, we explored the effects of in ovo L-Met injection on the intestinal development of chicks at E9. We demonstrate that L-Met promotes intestinal cell proliferation to improve intestinal epithelial integrity by activating the JAK2/STAT3 pathway. Our findings might provide a nutritional intervention for chick embryo growth involving JAK2/STAT3 signaling.

2. Materials and methods

2.1. Animal ethics

The experiments were approved by the Care and Use of Laboratory Animals Committee of South China Agricultural University (Guangzhou, Guangdong, China).

2.2. L-Met solutions and in ovo injection procedure

L-Met purchased from Sigma–Aldrich (purity ≥ 98%, St. Louis, MO, USA) was dissolved in phosphate-buffered saline (PBS) and filtered using a 0.22-μm syringe filter. The fertilized eggs of yellow-feathered broiler chicks, purchased from Qingyuan Chicken Company (Qingyuan, Guangdong, China), were incubated in an automatic hatcher (CFK, Keyu Incubation Equipment Company, Dezhou, Shandong). On E6 unfertilized and dead eggs were removed; then, a total of 80 eggs (45.03 ± 2.25 g) were selected and randomly divided into 4 treatments with 20 eggs per treatment. PBS (control, CON), 5, 10, or 20 mg L-Met was added to the eggs by in ovo injection at E9. The steps were as follows: firstly, a needle was used to make a small hole at 1/3 of the sharp end of the embryo egg after disinfection with a 75% alcohol-soaked cotton ball; secondly, 0.5 mL of the solution was injected into the yolk with a 1 mL disposable sterile syringe. After hatching, the chicks were sacrificed by CO2 inhalation followed by cervical dislocation to ensure death, then the intestinal samples were collected.

2.3. Hematoxylin and eosin (H&E) staining

After fixing with 4% paraformaldehyde, the jejenum samples were made into paraffin-embedded tissues for further histological

![Figure 1](https://example.com/figure1.png)

**Fig. 1.** The in ovo injection of methionine increases the small intestine weight of chicks. (A) Body weight. (B) Intestinal weight. (C) Intestinal length. Bars without a common letter indicate significant differences \((P < 0.05)\); the values are means ± SEM. \(n = 6\). CON – control.
processing. Serial sections of paraffin-embedded tissues with a thickness of 4 μm were cut using a microtome (Microm-HM340E, Thermo Fisher Scientific, Waltham, MA, USA) and stained with H&E. Then, images were captured under a microscope (Ti2-U, Nikon, Tokyo, Japan), and villus height and crypt depth were measured with Image-Pro Plus software.

2.4. Transepithelial electrical resistance (TEER)

To measure TEER, jejunum samples were balanced in Krebs–Ringer buffer (pH 7.4, 1.25 mmol/L NaCl, 2.5 mmol/L KCl, 1.25 mmol/L NaH2PO4, 2 mmol/L CaCl2, 1 mmol/L MgCl2, 25 mmol/L NaHCO3, 25 mmol/L glucose) for 10 min. Then, the samples were mounted directly onto and compressed between the two-chamber halves of an Ussing Chamber (Beijing Jingong Hongtai Technology CO., LTD., Beijing, China), representing the apical side and basolateral side, and surrounded by 7-mL Krebs–Ringer buffer on each side. The system was water-jacketed to 37 °C and carbonated with a carbogen (95% O2 and 5% CO2) gas flow. After an equilibration period of 30 min, the solutions were replaced with fresh Krebs–Ringer buffer, and then the experiments were run. Transepithelial electrical resistance (Ω/cm², resistance/surface area of the monolayer) was recorded as 3 consecutive measurements after subtracting the filter resistance value.

2.5. Scanning electron microscopy (SEM)

After fixing with 2.5% glutaraldehyde overnight, the jejuna were washed 3 times with PBS and treated with 1% osmium tetroxide for 1 h. Then, the jejuna were dehydrated with different concentrations of alcohol and dried to the critical point (EM CPD300, Leica Microsystems, Wetzlar, Germany), glued on stubs using conductive glue and coated with gold. Completely processed jejunum samples were examined using an EVO MA 15 SEM (Carl Zeiss, Jena, Germany).

2.6. Western blotting

Proteins were isolated from jejunum samples and analyzed as described previously (Xie et al., 2020). Total protein was extracted from the intestinal tissues using radioimmunoprecipitation assay lysis buffer, and the protein concentration in the homogenates was determined using the BCA protein assay kit (Thermo Fisher Scientific, Rockford, Illinois, USA). The proteins and color prestained protein marker (M222-10, GenStar, Beijing, China) were separated on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels and then transferred onto polyvinylidene fluoride membranes (Millipore, Billerica, Massachusetts, USA). The band densities were quantified using ImageJ.
software (version 1.8.0, National Institute of Health, Bethesda, MD, USA). The results were presented as the ratios of target protein level to β-actin level or the total protein level. The following antibodies were used: anti-p-JAK2 (#3771, CST, Cell Signaling Technology, Beverly, MA, USA), anti-JAK2 (#3230, CST), anti-p-STAT3 (#SC-8059, Santa Cruz Biotechnology, Dallas, TX, USA), anti-STAT3 (#SC-8019), anti-ZO-1 (#339100, Thermo Fisher), anti-Claudin-1 (#374900, Thermo Fisher), and anti-β-actin (#250132, ZENBIO, Chengdu, Sichuan, China).

2.7. Immunofluorescence

Immunofluorescence analysis was performed as described previously (Chen et al., 2020b). Fluorescence images were obtained with a microscope (NIS-Elements, Nikon, Tokyo, Japan). The following antibodies and reagents were used: anti-ZO-1, anti-claudin-1, and anti-Ki67 (#NB500-170, Novus Biologicals, Littleton, CO, USA), anti-Villin (#SC-58897, Santa Cruz Biotechnology); anti-p-JAK2, anti-p-STAT3, and FITC-conjugated secondary antibody (#115-545-003, Jackson Laboratory, Jackson, MS, USA); Cy3-conjugated secondary antibody (#111-165-045, Jackson Laboratory); and 4′,6-diamidino-2-phenylindole (DAPI, Sigma–Aldrich).

2.8. Statistics

The data were analyzed using SPSS software version 20 (SPSS Inc., Chicago, IL, USA). Duncan’s multiple range test was used to assess the differences between the 4 groups. P-values < 0.05 were considered statistically significant.

Fig. 3. The in ovo injection of methionine enhances the intestinal barrier function of chicks. (A) Transepithelial cell resistance (TEER) of the jejunum of chicks injected with phosphate-buffered saline (PBS) or L-methionine (L-Met). (B to C) The expression of ZO-1 and claudin-1 in the jejunum of chicks injected with PBS or L-Met. (D) Representative images of immunofluorescence (IF) staining with a ZO-1 antibody in the chick jejunum are shown (200× magnification; scale bar, 100 µm). (E) Statistical analysis of the fluorescence signal intensity based on the images shown in (D). (F) Representative images of IF staining with a claudin 1 antibody in the chick jejunum are shown (200× magnification; scale bar, 100 µm). (G) Statistical analysis of the fluorescence signal intensity based on the images shown in (F). Bars without a common letter indicate significant differences (P < 0.05); the values are means ± SEM. n = 3. CON = control.
3. Results

3.1. L-Met promotes intestinal development and epithelial integrity

To study the effect of in ovo L-Met injection on the intestinal development of chick embryos, we measured the weight of the duodenum, jejunum, and ileum on the day of hatching and examined the morphology of the villi by H&E staining and SEM. The results showed that compared with the CON group, the chicks injected with L-Met did not have significantly altered body weight or small intestine length ($P > 0.05$, Fig. 1A and B). In particular, compared with the CON group, in ovo injection of 10 and 20 mg L-Met significantly increased the duodenal weight ($P < 0.05$, Fig. 1C). The 3 doses of L-Met markedly increased the jejunal weight ($P < 0.05$, Fig. 1C). For the ileum, 20 mg of L-Met injection significantly increased its weight ($P < 0.05$, Fig. 1C). H&E staining showed that the injection of 5, 10, and 20 mg of L-Met into the yolk significantly increased the villus height and crypt depth ($P < 0.05$, Fig. 2A, B and C). Further, the structural integrity of villi in the jejunum in the 5 and 10 mg of L-Met groups was better than that in the other groups (Fig. 2D).

3.2. L-Met enhances intestinal barrier function

To examine whether injection of L-Met into eggs can enhance the intestinal barrier function in the chick embryo, the TEER values and the tight junction protein expression of ZO-1 and claudin1 were determined. As shown in Fig. 3A, compared with the CON group, the group injected with 10 mg of L-Met showed significantly increased TEER values ($P < 0.05$), and the group injected with 20 mg of L-Met showed significantly increased expression of the ZO-1 and claudin-1 in the jejunum ($P < 0.05$, Fig. 3B and C). Similarly, the fluorescence signals of ZO-1 and claudin-1 on the crypt–villus axis were higher in all groups injected with L-Met than in the CON group. These data indicated that in ovo injection of L-Met enhances intestinal barrier function.

3.3. L-Met promotes the proliferation and differentiation of intestinal cells

Next, we tested the effect of L-Met on the proliferation and differentiation of intestinal epithelial cells. Higher fluorescence signal intensities of Ki67 (labels proliferating cells) ($P < 0.05$, Fig. 4A...
and B) and villin (labels absorptive cells) ($P < 0.05$, Fig. 4C and D) were observed after injecting L-Met. These results suggested that L-Met promotes ISC division and improves ISC differentiation into absorptive cells.

3.4. L-Met potentiates JAK2/STAT3 signaling pathway activity

Finally, we explored the possible mechanism of L-Met in intestinal development. Western blotting (Fig. 5A and B) and immunofluorescence (Fig. 5C, D, and E) showed that the protein expression of p-JAK2 and p-STAT3 was significantly increased in the L-Met group ($P < 0.05$). The results suggested that L-Met potentiates the JAK2/STAT3 signaling pathway.

4. Discussion

The present research demonstrates that supplementation with L-Met in ovo improves the intestinal structure and barrier function of chick embryos. Mechanistically, enterocytes respond to the nutritional environment (L-Met) to drive their rapid proliferation and differentiation, which promotes the renewal of the intestinal epithelium. As early as 1999, Ohta et al. (1999) found that the injection of amino acids into the yolk at E7 can accelerate the development of embryos without affecting their hatchability. Our recent investigation confirmed that 10 mg L-Met significantly increased the diameter and density of feather follicles (Chen et al., 2020a). The present data further support the beneficial effects of L-Met on chick embryos, including increased intestinal weight. Generally, the weight of the small intestine is considered an important factor that reflects the development of the small intestine. Zhou et al. (2020) demonstrated that deoxynivalenol causes a significant decrease in jejunal weight accompanied by severe villous atrophy and intensive crypt loss. In the present study, the significant rise in villus height and crypt depth after L-Met injection suggests increasing the absorptive surface area and is closely correlated to the increase in the mass and numbers of enterocytes, which was supported by villin and Ki67 staining.

An intact intestinal epithelial structure indicates a strong barrier function and is composed of monolayer intestinal epithelial cells and tight junctions between enterocytes. These tight junctions form a selective barrier that allows the transcellular transport of various dietary nutrients from the lumen into the internal circulation and restricts the passage of harmful substances (Chelakkot et al., 2018). The assembly and coordination of diverse tight junctions are essential for guarding the paracellular pathway. In particular, the transmembrane proteins claudins are linked to the actomyosin fibers of the cytoskeleton by Zonula occludens (Zos) (Wang et al., 2015). A previous study showed that Met deficiency impaired the intestinal function of chicks (Ruan et al., 2018). The supplementation of L-Met reverses the inhibitory effect of deoxynivalenol on the expression of ZO-1 and claudin-1 in the mouse intestine (Zhou et al., 2019). The difference is that we found that L-Met might enhance tight junctions in the embryonic jejunum in chicks under normal conditions. Collectively, these findings imply that L-Met may play a dual role in stimulating intestinal epithelial replenishment under homeostasis and regeneration after injury.
It is worth mentioning that the integrity of the intestinal structure and barrier function mostly depends on the proliferation and differentiation of ISC (Qi et al., 2020). These cells divide rapidly while migrating along the crypt–villus axis. Ki67 is indispensable for cells in the G1, S, G2, and M phases and determines cell proliferation activity. Our results proved that L-Met could maintain the rapid turnover of intestinal epithelium by accelerating cell proliferation in chick embryos. Subsequently, ISC differentiate into cells of various lineages in the villi, including absorptive cells, which account for more than 90% of all cells (Zhou et al., 2020). Villin is widely known as an F-actin bundling protein involved in the maintenance of the brush border of absorptive cells, and it is a sign of intestinal cell differentiation and maturation (Nick et al., 2015). We identified that Villin is highly expressed after L-Met supplementation, suggesting that L-Met could promote ISC differentiation and enhance nutrient absorption. Intestinal stem cell expansion is cross-controlled by a variety of signal transduction pathways in the ISC niche, including Wnt, Notch, JAK/STAT, and mTORC, which together ensure that the intestine is in long-term homeostasis.

Richmond et al. (2018) demonstrated that ISC are initiated to proliferate and contribute to the subsequent regenerative response via the JAK/STAT signaling pathway. Furthermore, JAK/STAT3 activation strengthens intestinal epithelial barrier function (Kotelevets and Chastre, 2020; Zhao et al., 2020; Fang et al., 2019). Our present study indicated that L-Met could activate JAK2/STAT3 in the intestine of chick embryos; however, how to activate it is still unclear. There may be 3 ways to mediate this process, alone or in combination. The first is that L-Met enhances the assembly and secretion of cytokine ligands; the second is that L-Met directly binds to cytokine receptors; the third is that L-Met is transported to the cytoplasm and directly or indirectly modifies JAK2 itself or through its metabolites. The exploration of these problems will help to improve the signal network of embryonic intestinal development and advance the targeted regulation of functional amino acids.

In summary, through the chick embryo model, we found that in vivo injection of L-Met can enhance intestinal cell proliferation and differentiation to improve the structural and functional integrity of the intestinal epithelium through activation of the JAK2/STAT3 signaling pathway. Our results may provide new insights for regulating the intestinal development of embryonic chicks and the rapid growth of chicks after hatching.

Author contributions

Mengjie Chen: conceptualization, investigation, data curation, methodology, writing - original draft. Jiayi Zhou: methodology, data curation, writing - review & editing. Yijun Chen: validation, methodology. Xiuxi Wang: supervision. Huichao Yan: resources. Chunqi Gao: conceptualization, funding acquisition, project administration, writing - review & editing.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

Acknowledgments

This work was jointly supported by the National Natural Science Foundation of China (31972585) and the Technical System of Poultry Industry of Guangdong Province, China (2021KJ128).

References

Chelakkot C, Ghim J, Jyu SH. Mechanisms regulating intestinal barrier integrity and its pathological implications. Exp Mol Med 2018;50(8):1–9.

Chen MJ, Xie WY, Pan NX, Wang XQ, Yan HC, Gao CQ. Methionine improves feather follicle development in chick embryos by activating Wnt/b-catenin signaling. Poultry Sci 2020a;99:4479–87.

Chen MJ, Fa Z, Jiang SG, Wang XD, Yan HC, Gao CQ. Targeted disruption of TORC1 retards young squash growth by inhibiting the synthesis of crop milk protein in breeding pigeon (Columba livia). Poultry Sci 2020b;99(1):416–22.

Fang XY, Wu CQ, Wang QY, Tang JC. Farnesol contributes to intestinal epithelial barrier function by enhancing tight junctions via the JAK/STAT3 signaling pathway in differentiated Caco-2 cells. J Bioenerg Biomembr 2019;51:403–12.

Gilbert ER, Li H, Emmerson DA, Webb Jr KE, Wong EA. Developmental regulation of nutrient transporter and enzyme mRNA abundance in the small intestine of poultry. Poultry Sci 2007;86:1739–53.

Hou QH, Ye LL, Liu HF, Huang LL, Yang Q, Turne JR, et al. Lactobacillus accelerates ISC's regeneration to protect the integrity of intestinal mucosa through activation of STAT3 signaling pathway induced by LPLs secretion of IL-22. Cell Death Diff 2018;25(9):1657–70.

Jiang HQ, Patel PH, Kohlmaier A, Grenley MO, McEwen DG, Edgar BA. Cytokine/Jak/Stat signaling mediates regeneration and homeostasis in the Drosophila midgut. Cell 2009;137(7):1343–55.

Kadam MM, Barekatain MR, Bhanja SK, Iji PA. Prospects of in ovo feeding and nutrient supplementation for poultry: the science and commercial applications-A review. J Sci Food Agric 2013;93(15):3654–61.

Kotelevets L, Chastre L. Rac 1 signaling: from intestinal homeostasis to colorectal cancer metastasis. Cancers 2020;12(3):665.

Li H, Gilbert ER, Zhang Y, Crasta O, Emmerson D, Webb Jr KE, et al. Expression profiling of the solute carrier gene family in chicken intestine from the late embryonic to early post-hatch stages. Anim Genet 2008;39(4):407–24.

Morgan Jr ET. Nutrition of the developing embryo and hatchling. Poultry Sci 2007;86(5):1043–9.

Morikawa T, Baba Y, Yamauchi M, Kuchiba A, Nosho K, Shima K, et al. STAT3 expression, molecular features, inflammation patterns, and prognosis in a database of 724 colorectal cancers. Clin Canc Res 2011;17(6):1452–62.

Nick MS, Monica IC, Lisa AT, Boyd T, Kurek KC, Goldsmith JD, et al. Villin immunohistochemistry is a reliable method for diagnosing microvillus inclusion disease. Am J Surg Pathol 2015;39(2):245–50.

Obata F, Tsuda-Sakurai K, Yamazaki T, Nishio R, Nishimura K, Kimura M, et al. Nutritional control of stem cell division through S-adenosylmethionine in Drosophila intestine. Dev Cell 2018;44(6):741–51.

Ohto Y, Tsushima N, Koide K, Kida MT, Ishibashi T. Effect of amino acid injection in broiler breeder eggs on embryonic growth and hatchability of chicks. Poultry Sci 1999;78:1493–8.

Petersen LW, Arts J. Intestinal epithelial cells: regulators of barrier function and immune homeostasis. Nat Rev Immunol 2014;14(3):141–53.

Qi DJ, Shi WB, Adrian R, Kuss MA, Pang XN, He YN, et al. Repair and regeneration of small intestine: a review of current engineering approaches. Biomaterials 2020;240:119832.

Richmond CA, Bickner H, Shah MS, Ediger T, Deary L, Zhou F, et al. JAK/STAT1 signaling is required for reserve intestinal stem cell activation during intestinal regeneration following acute inflammation. Stem Cell Rep 2018;10(1):1–16.

Ruan T, Li L, Lyu Y, Luo Q, Wu BY. Effect of methionine deficiency on oxidative stress and apoptosis in the small intestine of broilers. Acta Vet Hung 2018;66(5):1–26.

Saito Y, Iwatsuki K, Hanayu H, Maruyama N, Aihara E, Tadaishi M, et al. Effect of essential amino acids on enteroids: methionine deprivation suppresses proliferation and affects differentiation in enteroid stem cells. Biochem Biophys Res Commun 2017;488(1):171–6.

Shiraki N, Shiraki Y, Tsuyama T, Obata F, Miura M, Nagae G, et al. Methionine metabolism regulates maintenance and differentiation of human pluripotent stem cells. Cell Metab 2014;19(5):780–94.

Southwell BR. Staging of intestinal development in the chick embryo. Anat Rec A Discov Mol Cell Evol Biol 2006;288(8):909–20.

Tang YY, Tong XH, Li Y, Jiang GJ, Yu ML, Chen YL, et al. JAK2/STAT3 pathway is involved in the protective effects of edemical growth factor receptor activation against cerebral ischemia/reperfusion injury in rats. Neurosci Lett 2018;662:219–26.
Vieira SL, Lemme A, Goldenberg DB, Brugalli I. Responses of growing broilers to diets with increased sulfur amino acids to lysine ratios at two dietary protein levels. Poultry Sci 2004;83:1307–13.

Wang B, Wu GQ, Zhou ZQ, Dai ZL, Sun YL, Ji Y, et al. Glutamine and intestinal barrier function. Amino Acids 2015;47(10):2143–54.

Wang WW, Qiao SY, Li DF. Amino acids and gut function. Amino Acids 2009;37(1):105–10.

Willemsen H, Debonne M, Swennen Q, Everaert N, Careghi C, Han H, et al. Delay in feed access and spread of hatch: importance of early nutrition. World Poultry Sci J 2010;66(2):177–88.

Xie WY, Chen MJ, Jiang SC, Wang QY, Yan HC, Gao CQ. The Wnt/β-catenin signaling pathway is involved in regulating feather growth of embryonic chicks. Poultry Sci 2020;99:2315–23.

Yu H, Pardoll D, Jove R. STATs in cancer inflammation and immunity: a leading role for STAT3. Nat Rev Canc 2009;9(11):798–809.

Zhao Q, Xing F, Tao YY, et al. Xiaozhang Tie improves intestinal motility in rats with cirrhotic ascites by regulating the stem cell factor/c-kit pathway in interstitial cells of Cajal. Front Pharmacol 2020;11:1.

Zhou JY, Lin HL, Wang Z, Zhang SW, Huang DG, Gao CQ, et al. Zinc L-aspartate enhances intestinal stem cell activity to protect the integrity of the intestinal mucosa against deoxynivalenol through activation of the Wnt/β-catenin signaling pathway. Environ Pollut 2020;262:114290.

Zhou JY, Wang Z, Zhang SW, Lin HL, Gao CQ, Zhao JC, et al. Methionine and its hydroxyl analogues improve stem cell activity to eliminate deoxynivalenol-induced intestinal injury by reactivating Wnt/β-catenin signaling. J Agric Food Chem 2019;67:11464–73.