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Basic nutritional investigation

L-Threonine improves intestinal mucin synthesis and immune function of intrauterine growth–retarded weanling piglets

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ABSTRACT

Objectives: The aim of this study was to investigate the effects of dietary L-threonine supplementation on the growth performance, intestinal immune function, mucin synthesis, and goblet cell differentiation in weanling piglets with intrauterine growth retardation (IUGR).

Methods: Eighteen litters of newborn piglets were selected at birth, with one normal birthweight (NBW) and two IUGR piglets in each litter. At weaning, the NBW piglet and one of the IUGR piglets were assigned to groups fed a basal diet (i.e., the NBW-CON and IUGR-CON groups). The other IUGR piglet was assigned to a group fed the basal diet supplemented with 2 g L-threonine per kg of diet (i.e., IUGR-Thr group). Therefore, all piglets were distributed across three groups for a 3-wk feeding trial.

Results: Compared with NBW, IUGR decreased growth performance, increased ileal proinflammatory cytokine levels, and reduced ileal mucin 2 (Muc2) content and goblet cell density of weanling piglets. Supplementation of L-threonine increased the feed efficiency of the IUGR-Thr group compared with the IUGR-CON group. The L-threonine–supplemented diet attenuated ileal inflammatory responses of the IUGR-Thr piglets and increased production of Muc2 and secretory immunoglobulin A and density of goblet cells. In addition, L-threonine supplementation downregulated δ-like 1 and hes family bHLH transcription factor 1, whereas growth factor independence 1 and Kruppel-like factor 4 expression levels were upregulated.

Conclusion: Dietary L-threonine supplementation attenuates inflammatory responses, facilitates Muc2 synthesis, and promotes goblet cell differentiation in the ileum of IUGR piglets.

Introduction

Intrauterine growth retardation (IUGR) is usually ascribed to chronic placental insufficiency, which is a common complication of pregnancy that impairs nutrient transport from mother to fetus [1]. As an adaptation to placental insufficiency, redistribution of cardiac output in the fetus maintains substrate supply to key organs (heart and brain) at the expense of visceral organs, such as the liver and small intestine [1]. Such a defect can disrupt the normal trajectory of immune maturation, negatively affecting the composition and secretory products of the intestinal epithelial surface [2]. Emerging evidence indicates that a delay in the development of a mucus barrier and a lower capacity of goblet cells to produce mucus as a response to infection may explain why IUGR neonates are more susceptible to intestinal diseases [3]. The mucus layer coating the intestinal epithelium forms a physical barrier to protect against endogenous and exogenous irritants and microbial attachments and invasions [4]. Clinically, the mucus layer is relatively deficient in premature infants possibly because of the decrease of goblet cell numbers, which may increase the propensity for adherence of pathogenic organisms and their subsequent translocation [5]. Thus, the disruption in the mucus barrier may be responsible for a default in the mucosal protection, rendering the IUGR intestine susceptible to injury, infection, and inflammation [6].

A potential mechanism for the impaired mucus barrier of IUGR animals may be associated with the limited nutrient availability. Of

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particular interest is threonine, which is an essential amino acid that is abundantly present in intestinal mucins. The intestine utilizes threonine more than other essential amino acids, and the gut retains as much as 60% of dietary L-threonine for the synthesis of mucins [7]. If dietary L-threonine intake is deficient, muscle growth and the functions of other tissues of piglets are likely compromised at the expense of maintaining the mucus layer in mucin-producing tissues [8]. In that regard, piglets are very sensitive to threonine deficiencies that could impair gut mucin synthesis and barrier function. It has been reported that IUGR piglets exhibited a decreased availability of threonine for intestinal mucin synthesis in early life [9], which may provide a potential explanation for their increased risk for intestinal diseases [3]. Thus, a substantial and constant supply of L-threonine is necessary to maintain the intestinal health of IUGR animals. However, to our knowledge, no sufficient information is available on the intervention effect and relevant mechanism.

In the present study, we investigated whether dietary supplementation with L-threonine could improve intestinal immune function, mucin synthesis, and goblet cell differentiation of weanling piglets subjected to IUGR, which may help to establish appropriate nutrition strategies to optimize their growth performance. Importantly, the pig probably provides the optimal animal model for humans to study the development of gastrointestinal tract structure and function in IUGR syndrome [10]. The results of medical testing on pigs are regarded as having a high positive predictive value for subsequent translation to humans. Therefore, this study may have implications for the treatment of neonatal intestinal diseases in the IUGR offspring.

Materials and methods

Experimental design, diets, and management

All animal procedures were undertaken in accordance with the guidelines of the Institutional Animal Care and Use Committee of Nanjing Agricultural University.

Ninety-three healthy sows with similar parity (second or third) and similar expected farrowing dates were chosen for inclusion in the study. Eighteen litters of between 10 and 13 piglets were selected at birth, and two males in each litter met the selection criteria for IUGR. Newborn piglets (Duroc × [Landrace × Yorkshire]) with a birthweight (BW) close to the average BW of the herd (i.e., within 0.5 SD) were identified as normal BW (NBW), and those with a 2-SD lower BW were defined as IUGR [11,12]. Two IUGR male piglets (0.90 ± 0.05 kg) and one NBW male piglet (1.52 ± 0.04 kg) were selected from each litter. All piglets were weaned at 21 d and transferred to the weaning unit. In each litter, the NBW piglet and one of the IUGR piglets were assigned to groups fed the basal diet (the NBW-CON and the IUGR-CON group, respectively). The other IUGR piglet was assigned to the group fed the basal diet supplemented with 2 g/kg L-threonine (the IUGR-Thr group; Fig. 1). The piglets remained in these three groups for a 3-wk period and each group consisted of six replicates with three piglets per replicate. The L-threonine used in this study was purchased from Sigma-Aldrich (St. Louis, MO, USA) and had a purity of 99%. Two isonitrogenous and isoenergetic diets were formulated according to the recommended nutrient requirements of National Research Council (2012), except for threonine [13], and L-alanine was added to the basal diet to obtain the same levels of total nitrogen as the L-threonine–supplemented diet. The ingredient composition and nutrient content of the diets are shown in Supplementary Table 1. Piglets had free access to food and water during the feeding period, and their average daily gain (ADG) and average daily feed intake (ADFI) were recorded every week. In addition, the feed efficiency (FE) was calculated by dividing ADG by ADFI.

Sample collection

At the end of the experiment, the piglet with a BW closest to the mean BW of its replicate was selected. Detailed procedures for the collection of heparinized blood and ileal segment and mucosa samples in this study are available in the supplementary information.

Measurement of plasma immunoglobulin contents and ileal immune status

Plasma immunoglobulin (Ig) G, IgA, and IgM concentrations and ileal secretory immunoglobulin A (sIgA) and mucin 2 (Muc2) contents were measured using porcine-specific enzyme-linked immunosorbent assay kits (CUSABIO Biotech, Wuhan, China). Detailed information about the detection range, minimum detectable dose, and inter- and intraassay coefficients of variance of each kit can be found in the supplementary information. In addition, the levels of cytokines (tumor necrosis factor [TNF]-α, interferon [IFN]-γ, interleukin [IL]-1 β, IL-4, IL-6, and IL-10) in the ileal mucosa were determined using the ProcartaPlex multiplex immunoassay (Luminex, Austin, TX, USA) kit according to the manufacturer's instructions obtained from Affymetrix eBioscience (Santa Clara, CA, USA).

Goblet cell staining

The combined Alcian Blue/periodic acid Schiff stain technique was then employed to measure the intestinal goblet cell density. Goblet cells were counted in 15 well-oriented villi per section using the Nikon ECLIPSE 80 i light microscope (Nikon Corporation, Tokyo, Japan). Goblet cell density was calculated as the goblet cell count divided by the corresponding villus length and averaged and expressed as goblet cell numbers per 100 μm of villus length.

Total RNA isolation and quantitative real-time PCR analysis

The methodologies of the total RNA isolation and the determination of transcriptional activities of the target and reference genes (δ-like 1 [*Dll1*], hes family bHLH transcription factor 1 [*Hes1*], atonal bHLH transcription factor 1 [*Math1*], growth factor independence 1 [*Gfi1*], Kruppel-like factor 4 [*Klf4*], E74-like factor 3 [*Elf3*], galactose-3-*O*-sulfotransferase [*GAL3 ST*] 2, *GAL3 ST4*, *Muc2*, glyceraldehyde-3-phosphate dehydrogenase, and *β-actin*) are supplemented in the supplementary information.

Statistical analysis

Data were tested for normality (Shapiro–Wilk test) and homogeneity of variances (Levene's test) before statistical analysis. Heterogeneous data (i.e., plasma IgA content and ileal *Gfi1* mRNA abundance), or data not normally distributed (i.e.,

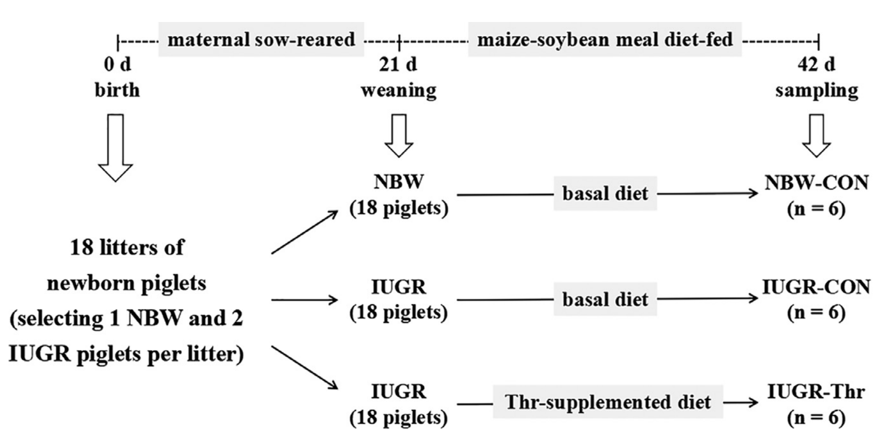

Fig. 1. Schematic representation of the experimental procedures.

Table 1
Effects of dietary L-threonine supplementation on growth performance of weanling piglets with IUGR

Items	NBW-CON (NC group)	IUGR-CON (IC group)	IUGR-Thr (IT group)	*P*-value		
				Overall	NC vs IC	IC vs IT
ADG (g/d)	336.51 ± 13.38	221.16 ± 12.96	260.32 ± 13.31	<0.001	<0.001	0.125
ADFI (g/d)	486.24 ± 17.39	344.71 ± 15.86	369.84 ± 11.84	<0.001	<0.001	0.489
FE (g/g)	0.69 ± 0.02	0.64 ± 0.01	0.70 ± 0.02	0.034	0.085	0.040

ADFI, average daily feed intake; ADG, average daily gain; FE, feed efficiency.; IC, IUGR-CON; IT, IUGR-Thr; IUGR-CON, intrauterine growth retardation control; IUGR-Thr, intrauterine growth retardation threonine; NBW-CON, normal birthweight control; NC, NBW-CON.

ileal IL-4 content), were analyzed using the non-parametric Kruskal–Wallis test. Pairwise differences in rank sums were evaluated using selected comparisons tests, and all normal data were tested for statistical significance using one-way analysis of variance and Tukey's post hoc test for pairwise comparisons. Using SPSS version 22 (SPSS Inc., Chicago, IL, USA), data were analyzed, and $P < 0.05$ was considered statistically significant. Results are presented as means with SE.

Results

Growth performance

Piglets in the IUGR-CON group exhibited compromised growth performance during the first 3 wk of postweaning compared with the NBW-CON group, as marked by the decreases in ADG ($P <$ 0.001) and ADFI ($P < 0.001$; Table 1). Increasing dietary L-threonine provision did not affect ADG and ADFI ($P > 0.05$); it increased FE ($P = 0.040$) of the IUGR-Thr piglets compared with the IUGR-CON piglets.

Plasma Ig contents

Compared with the NBW-CON group (Table 2), IUGR had no effect on plasma concentrations of the IgG, IgA, or IgM of weanling piglets ($P > 0.05$). A higher L-threonine diet significantly elevated the content of IgG ($P = 0.015$) in the plasma of the IUGR-Thr group compared with the IUGR-CON group. However, supplementation with L-threonine did not alter the plasma levels of circulatory IgA or IgM ($P > 0.05$).

Ileal cytokine contents

Compared with NBW (Table 3), IUGR significantly increased the contents of TNF-α ($P = 0.010$), IFN-γ ($P = 0.028$), and IL-1 β ($P = 0.002$) in the ileum of the IUGR-CON group. Conversely, a decreased concentration of ileal TNF-α ($P = 0.011$) was observed in the IUGR piglets fed a higher L-threonine diet compared with those fed a basal diet. However, neither IUGR nor dietary L-threonine levels affected the levels of IL-4, IL-6, and IL-10 in the ileum ($P > 0.05$).

Ileal goblet cell density and Muc2 and sIgA concentrations

The IUGR-CON piglets showed decreased goblet cell density ($P = 0.019$) and Muc2 concentration ($P = 0.001$) in the ileum compared with the NBW-CON piglets (Table 4; Fig. 2). However, dietary L-threonine supplementation improved the numbers of goblet cells ($P = 0.002$) and increased the contents of Muc2 ($P = 0.041$) and sIgA ($P = 0.031$) in the ileum of the IUGR-Thr group compared with the IUGR-CON group.

Gene expression related to goblet cell differentiation

IUGR increased *Dll1* ($P = 0.014$) but decreased *Muc2* ($P = 0.034$) expression levels in the ileum of the IUGR-CON piglets compared with the NBW-CON piglets (Fig. 3). However, the mRNA levels of intestinal *Hes1, Math1, Gfi1, Klf4*, and *Elf3* were not affected by IUGR ($P > 0.05$). In addition, a higher L-threonine diet significantly downregulated the expression of *Dll1* ($P = 0.006$) and *Hes1* ($P = 0.004$), whereas it upregulated the abundance levels of *Gfi1* ($P = 0.006$), *Klf4* ($P = 0.019$), and *Muc2* ($P = 0.005$) compared with the basal diet. Dietary L-threonine levels had no effect on *Math1, Elf3, GAL3 ST2*, or *GAL3 ST4* mRNA levels ($P > 0.05$).

Discussion

Dietary threonine deficiency leads to intestine-related consequences, including diarrhea [14], compromised intestinal protein synthesis [15], ileal villus atrophy [16], and inferior mucin secretion [14,15]. Studies in animal models and humans demonstrated that under various pathologic conditions, such as ileitis [17], sepsis [18], colonic carcinoma [19], and premature delivery [20], the threonine requirement by the intestine is strongly increased because of the enhanced synthesis of secretory glycoproteins. As a result, a supply of L-threonine in regular diets designed for healthy individuals is likely inadequate for the maintenance of gut mucin synthesis and mucosal barrier function. This study demonstrates that supplementation of L-threonine improves the FE and intestinal immune function of the IUGR piglets during early life, which may be associated with the beneficial action of L-threonine administration to increase substrate availability for the synthesis of Muc2 and sIgA and facilitate the differentiation of goblet cells in the ileum.

It has been reported that IUGR piglets showed compromised growth performance compared with NBW piglets during the initial weeks after weaning [21]. The present study shows that the impairment of intestinal immune homeostasis or excessive inflammatory responses may be a potential explanation for the decreases

Table 2
Effects of dietary L-threonine supplementation on plasma immunoglobulin levels of weanling piglets with IUGR (μg/mL)

Items	NBW-CON (NC group)	IUGR-CON (IC group)	IUGR-Thr (IT group)	*P*-value		
				Overall	NC vs IC	IC vs IT
IgG	9968.52 ± 528.48	7744.31 ± 423.80	11350.54 ± 1187.80	0.018	0.148	0.015
IgA	640.81 ± 124.51	465.54 ± 97.00	570.49 ± 67.84	0.484	NS*	NS*
IgM	81.90 ± 9.47	109.60 ± 10.08	106.02 ± 12.18	0.169	0.190	0.969

IC, IUGR-CON; Ig, immunoglobulin; IT, IUGR-Thr; IUGR-CON, intrauterine growth retardation control; IUGR-Thr, intrauterine growth retardation threonine; NBW-CON, normal birthweight control; NC, NBW-CON; NS, non-significant.
*Non-significant values after Kruskal–Wallis comparison test.

Table 3
Effects of dietary L-threonine supplementation on ileal cytokine contents of weanling piglets with IUGR (pg/mg protein)

Items	NBW-CON (NC group)	IUGR-CON (IC group)	IUGR-Thr (IT group)	*P*-value		
				Overall	NC vs IC	IC vs IT
TNF-α	1.71 ± 0.19	2.58 ± 0.18	1.72 ± 0.17	0.005	0.010	0.011
IFN-γ	2.00 ± 0.18	2.94 ± 0.27	2.26 ± 0.22	0.030	0.028	0.126
IL-1 β	6.05 ± 0.51	9.53 ± 0.73	7.50 ± 0.50	0.003	0.002	0.069
IL-4	1.36 ± 0.08	1.27 ± 0.13	1.18 ± 0.16	0.185	NS*	NS*
IL-6	2.16 ± 0.17	2.76 ± 0.39	2.59 ± 0.49	0.519	0.508	0.945
IL-10	19.78 ± 1.38	14.80 ± 2.06	17.69 ± 1.50	0.142	0.123	0.460

IC, IUGR-CON; IFN, interferon; IL, interleukin; IL, interleukin; IT, IUGR-Thr; IUGR-CON, intrauterine growth retardation control; IUGR-Thr, intrauterine growth retardation threonine; NBW-CON, normal birthweight control; NC, NBW-CON; NS, non-significant; TNF, tumor necrosis factor.
*Non-significant values after Kruskal–Wallis comparison test.

in ADG and ADFI of the IUGR piglets, probably because of the diversion of available nutrients away from growth to support immune-related processes and synthesis of various mediators, such as cytokines. The IUGR-CON piglets exhibited elevated concentrations of ileal TNF-α, IFN-γ, and IL-1 β, supporting recent findings of a study conducted by Wang et al. [22], who found that the mRNA abundance of TNF-α was higher in the small intestine of IUGR piglets than in the NBW piglets at the end of suckling period. Interestingly, TNF-α was lower in the IUGR piglets at birth in that study, and this rapid increase of TNF-α transcriptional activity after birth indicates a high load of antigens in the intestine of IUGR piglets, which is capable of triggering a chronic inflammatory process [22].

Continuous overproduction of proinflammatory cytokines in response to inflammatory stimuli can aggravate the depletion of goblet cells, especially when the self-adjustment of the intestinal immune system is disturbed or the substrates of mucin synthesis cannot be replenished promptly. In the present study, IUGR was found to decrease the gene expression and protein synthesis of ileal Muc2 of weanling piglets. Damage to the epithelium, in particular, those events affecting the protective properties as offered by the secretory products of the goblet cells, is a likely cause of the inflammation [23]. The decrease of Muc2 synthesis would stimulate the release of more inflammatory mediators, thereby perpetuating a vicious cycle of Muc2 deficiency and inflammation. In fact, loss of Muc2 in the intestine can negatively affect mucus composition and then impair the epithelial barrier, resulting in an abnormal morphology and inflammation [23]. Thus, defects in mucin synthesis and gene expression may explain the increased incidence of intestinal inflammation in premature infants.

TNF-α can upregulate Muc2 transcription through activation of nuclear factor κ-B signals [6]. However, in the IUGR-CON piglets, the overproduction of TNF-α failed to stimulate the production of ileal Muc2, but it was still lower than in the NBW-CON piglets. In addition to the decreased supply of L-threonine, the maturity of the gut may be another explanation. McElroy et al. [5] found that TNF-α induced an increase in mucus secretion and a compensatory upregulation of Muc2 only in the mature ileum of mice; however, in the immature ileum, it caused a loss of mucus-containing goblet cells. These results once again confirmed the impaired capacity of IUGR piglets to regulate their immune homeostasis in the intestine and showed if the inflammatory process was not regulated promptly, it would have adverse consequences for growth and health status.

The present results show that feeding the IUGR piglets with a higher L-threonine diet may be a feasible approach to improving their growth performance and intestinal immune function. After a 3-wk feeding period, dietary L-threonine supplementation did not affect the ADG and ADFI of IUGR piglets, but improved their FE. These findings are similar to the results described in a pig model of systemic immune stress, in which dietary L-threonine levels increased from 8.5 to 9 g/kg and tended to increase the FE of weanling piglets during a 12- or 13-d trial [24]. However, Ren et al. [25] reported that the feed conversion ratio of normal piglets fed a diet containing 12.4 g/kg L-threonine was similar to that of their counterparts receiving the 8.4 g/kg L-threonine diet. These discrepancies among studies in their conclusions regarding the effect of extra L-threonine supplementation on the FE of weanling piglets are most likely to result from a combination of differences in dietary L-threonine levels, feeding periods, genetic background, and physiological status of the pigs.

The availability of dietary L-threonine affects plasma antibody production and whole-body immune function [26]. The present study found that increasing dietary L-threonine inclusion amplified the contents of plasma IgG and ileal sIgA of the IUGR-Thr piglets, suggesting that a higher inclusion of dietary L-threonine is beneficial to the production of Igs whose role is fundamental to the function of immune system. The requirement of threonine to maximize the humoral antibody and IG production of piglets is actually higher than that to maximize their growth performance [26].

Threonine is also of great importance for the maintenance of gut immune homeostasis of the IUGR piglets. A potential explanation may be that the L-threonine–supplemented diet increased the production of ileal sIgA, which is capable of protecting the intestinal epithelium from enteric pathogens and toxins, quenching bacterial virulence factors directly, and preventing complement activation and inflammatory responses to nonpathogenic antigens. Moreover, feeding the IUGR piglets a higher L-threonine diet for 3 wk yielded higher ileum Muc2 at both mRNA and protein levels

Table 4
Effects of dietary L-threonine supplementation on ileal goblet cell density and mucin 2 and secretory immunoglobulin A contents of weanling piglets with IUGR

Items	NBW-CON (NC group)	IUGR-CON (IC group)	IUGR-Thr (IT group)	*P*-value		
				Overall	NC vs IC	IC vs IT
Goblet cell density (n/100 µm villus height)	4.11 ± 0.28	3.11 ± 0.18	4.49 ± 0.21	0.002	0.019	0.002
Muc2 (ng/mg protein)	16.67 ± 1.65	6.23 ± 0.70	12.57 ± 2.24	0.002	0.001	0.041
sIgA (µg/mg protein)	1.11 ± 0.10	1.22 ± 0.24	2.07 ± 0.25	0.011	0.919	0.031

IC, IUGR-CON; IT, IUGR-Thr; Muc2, mucin 2; IUGR-CON, intrauterine growth retardation control; IUGR-Thr, intrauterine growth retardation threonine; NBW-CON, normal birthweight control; NC, NBW-CON; sIgA, secretory immunoglobulin A.

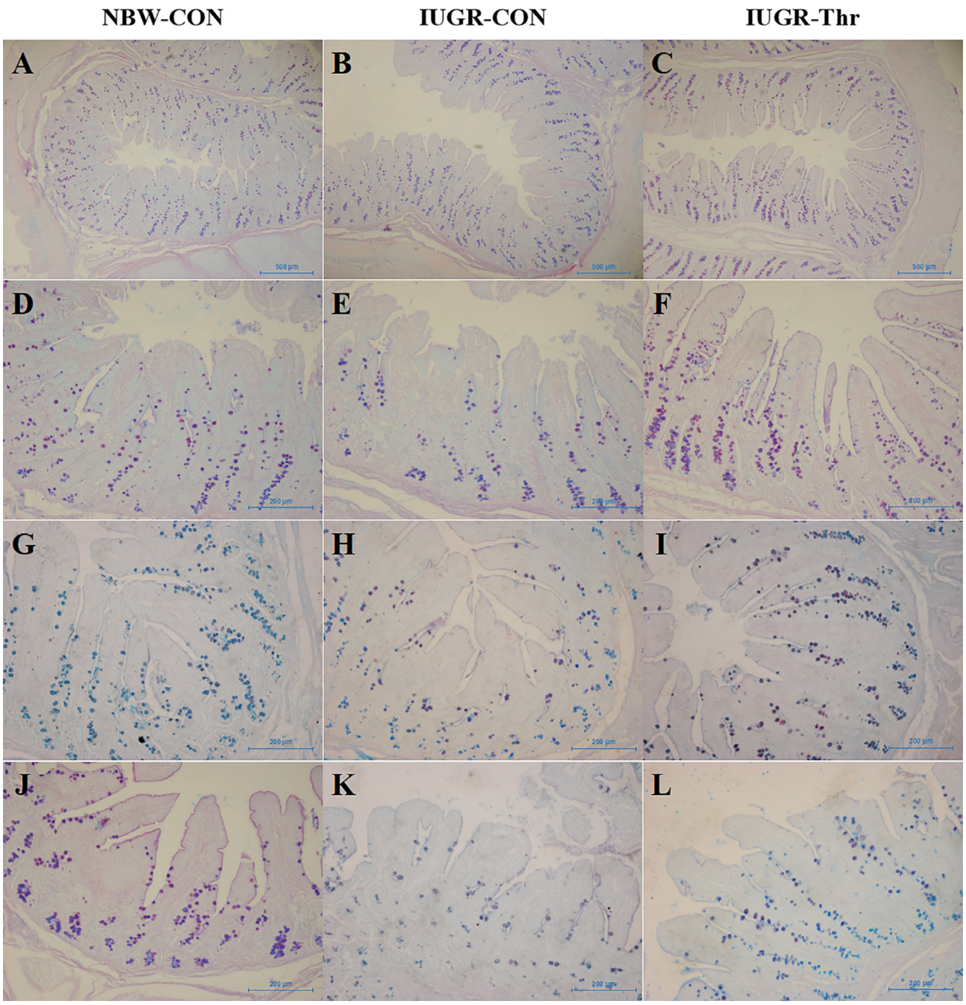

Fig. 2. Representative micrographs of goblet cell staining carried out on paraformaldehyde-fixed sections from the ileum (**A**–**C**, 40 × magnification; **D**–**L**, 100 × magnification).

than the basal diet, suggesting that L-threonine supplementation mitigated the low availability of threonine for intestinal mucin synthesis in the IUGR piglets [9]. Muc2 serves as an important constituent of the mucus layer and provides a physical barrier, facilitates removal of adherent bacteria, and concentrates enzymes near the epithelial surface to aid in host nutrient digestion [5]. These findings together with the increased sIgA production indicate that a higher L-threonine diet is beneficial to improve the innate intestinal defense and immune function of the IUGR piglets.

The present study found that the mechanism by which L-threonine supplementation stimulated ileal Muc2 synthesis may also be involved in the improved development of goblet cells. Increasing dietary L-threonine provision elevated goblet cell density in the ileum of the IUGR-Thr piglets, which may be attributed to the

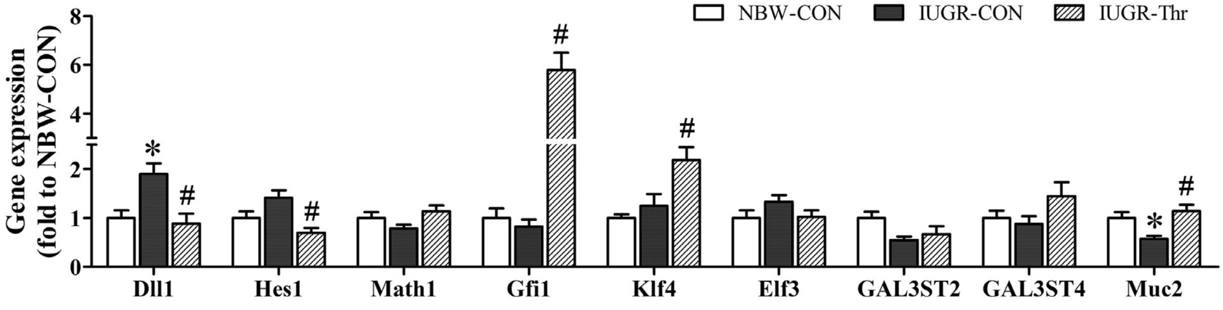

Fig. 3. Effects of dietary L-threonine supplementation on ileal gene expression of weanling piglets with intrauterine growth retardation. Values are expressed as the mean and SEM (n = 6). *Dll1*, delta-like 1; *Elf3*, E74-like factor 3; *GAL3 ST2*, galactose-3-*O*-sulfotransferase 2; *GAL3 ST4*, galactose-3-*O*-sulfotransferase 4; *Gfi1*, growth factor independence 1; *Hes1*, hes family bHLH transcription factor 1; IUGR-CON, intrauterine growth retardation control; IUGR-Thr, intrauterine growth retardation threonine; *Klf4*, Kruppel-like factor 4; *Math1*, atonal bHLH transcription factor 1; *Muc2*, mucin 2; NBW-CON, normal birthweight control. *Significant difference between NBW-CON and IUGR-CON groups ($P < 0.05$). †Significant difference between IUGR-CON and IUGR-Thr group ($P < 0.05$).

lower expression levels of *Dll1* and *Hes1*. A study conducted by Pellegrinet et al. [27] identified *Dll1* as being a key physiological ligand mediating Notch signaling in the intestinal epithelium of the mouse. As a result, the downregulation of *Dll1* expression decreases the signaling activity of the Notch pathway [27], which has a central role in regulating the secretory-absorptive cell fate decision during intestinal development. In the intestinal epithelium, inhibiting the Notch signal would lead to a massive conversion of epithelial cells into goblet cells, in conjunction with *Hes1* underexpression [28]. *Hes1* is a bHLH-type transcriptional repressor whose expression is transcriptionally activated by the Notch signal. Targeted inactivation of *Hes1* could induce an increase in secretory lineage but a decrease in the absorptive lineage of the intestine [29]. Thus, the greater numbers of goblet cells observed in the IUGR-Thr group may be partly due to the inhibitory effect of L-threonine supplementation on the Notch signal.

Math1 is regulated negatively by *Hes1*, and they play opposite roles in the cell choice between absorptive and secretory fate [30]. *Math1* expression is necessary for the development of the intestinal secretory cell lineage cells because Math1$^{-/-}$ mice are populated only by absorptive enterocytes [31]. In the present study, the IUGR-Thr group showed a numerically increased expression of *Math1* ($P = 0.108$) and significantly higher mRNA levels of *Gfi1* and *Klf4* than the IUGR-CON group. *Math1* functions as an amplifier for the Notch-Hes1-Math1 signaling cascade during the cell fate decision [30]. Any tiny fluctuation on the *Math1* expression may give rise to dramatic changes on the transcriptional activity of its downstream targets. *Gfi1* acts downstream of *Math1* and regulates the expression of *Klf4* directly, which is responsible for the differentiation of goblet cells [32]. Altogether, these observations indicate that improving the availability of dietary L-threonine for the intestine could attenuate the decreased numbers of goblet cells in the IUGR piglets probably by regulating the expression of genes involved in the Notch-Hes1-Math1 pathway.

Conclusion

The present results suggested that dietary L-threonine supplementation has beneficial effects on the intestinal immune homeostasis, mucin synthesis, and goblet cell differentiation of IUGR piglets. More importantly, this study may have the auxiliary preventive potential to treat intestinal disorders of IUGR infants in early life.

Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.nut.2018.07.114.

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