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Gene Expression Is Altered in Piglet Small Intestine by Weaning and Dietary Glutamine Supplementation[1–3]

Junjun Wang,[4,5] Lixiang Chen,[5–7] Peng Li,[5] Xilong Li,[5] Huaijun Zhou,[5] Fenglai Wang,[4]* Defa Li,[4] Yulong Yin,[5,6]* and Guoyao Wu[4–6]*

[4]State Key Laboratory of Animal Nutrition, China Agricultural University, Beijing, China 100094; [5]Texas Agricultural Experiment Station, The Texas A&M University System, College Station, TX 77843; [6]Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha, Hunan, China 410128; and [7]College of Animal Science and Technology, Hunan Agricultural University, Changsha, Hunan, China 410128

Abstract

Dietary supplementation of glutamine prevents intestinal dysfunction and atrophy in weanling piglets, but the underlying mechanism(s) are largely unknown. This study was conducted to test the hypothesis that weaning or glutamine may modulate expression of genes that are crucial for intestinal metabolism and function. In Expt. 1, we obtained small intestine from 28-d-old pigs weaned at 21 d of age and from age-matched suckling piglets. In Expt. 2, piglets were weaned at 21 d of age and then had free access to diets supplemented with 1% L-glutamine (wt:wt) or isonitrogenous L-alanine (control). At d 28, we collected small intestine for biochemical and morphological measurements and microarray analysis of gene expression using the Operon Porcine Genome Oligo set. Early weaning resulted in increased (52–346%) expression of genes related to oxidative stress and immune activation but decreased (35–77%) expression of genes related to macronutrient metabolism and cell proliferation in the gut. Dietary glutamine supplementation increased intestinal expression (120–124%) of genes that are necessary for cell growth and removal of oxidants, while reducing (34–75%) expression of genes that promote oxidative stress and immune activation. Functionally, the glutamine treatment enhanced intestinal oxidative-defense capacity (indicated by a 29% increase in glutathione concentration), prevented jejunal atrophy, and promoted small intestine growth (+12%) and body weight gain (+19%) in weaned piglets. These findings reveal coordinate alterations of gene expression in response to weaning and aid in providing molecular mechanisms for the beneficial effect of dietary glutamine supplementation to improve nutrition status in young mammals. J. Nutr. 138: 1025–1032, 2008.

Introduction

Recent studies have identified high concentrations of free glutamine (0.5–2 mmol/L) as well as peptide-bound glutamine plus glutamate in the milk of mammals, including pigs (1,2). This conditionally essential amino acid is a major energy substrate for rapidly dividing cells [including enterocytes and lymphocytes (3)]. It is also required for the synthesis of purine and pyrimidine nucleotides that are essential for the proliferation of cells, including intestinal mucosal cells and intraepithelial lymphocytes (4). Additionally, glutamine is a major substrate for the endogenous synthesis of arginine in most mammals (including humans and pigs) via the intestinal-renal axis (5). This synthetic pathway compensates for a marked deficiency of arginine (an essential amino acid for neonates) in milk during the suckling period (6). Furthermore, glutamine is required for the synthesis of *N*-acetylglucosamine-6-phosphate, a common substrate for the synthesis of glycoproteins that are particularly rich in intestinal mucosa (7).

Weaning is associated with reduced food consumption by young mammals, including piglets (8), therefore decreasing the intake of glutamine from the diet. Notably, weanling piglets often experience intestinal dysfunction and atrophy (9). Recent work has shown that dietary supplementation with glutamine prevents these gut problems (10). However, the underlying mechanism(s) are largely unknown. We hypothesized that weaning or glutamine may modulate expression of genes that are crucial for intestinal metabolism and function. This hypothesis was tested in the present study using microarray technology,

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[3] Supplemental Table 1 is available with the online posting of this paper at jn.nutrition.org.
* To whom correspondence should be addressed: g-wu@tamu.edu, wangfl@mafic.ac.cn, or yinyulong@isa.ac.cn.

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which provides a powerful discovery tool to simultaneously analyze expression of thousands of genes in a tissue (11).

Materials and Methods

All animals used in this study were humanely managed according to the established guidelines of the USDA. The experimental protocol was approved by the Texas A&M University Institutional Animal Care and Use Committee.

Animals and tissue collection. Pregnant sows were fed daily a 2-kg gestating diet during the entire period of pregnancy that met the NRC-recommended requirements for nutrients (12). After farrowing, sows had free access to a corn- and soybean meal-based diet that also met NRC-recommended requirements (13). Each sow freely nursed 9 piglets before weaning at 21 d of age. All sows had free access to drinking water during gestation and lactation periods. Neonatal piglets were used in 2 series of experiments.

In Expt. 1, 24 21-d-old piglets with similar body weights from 6 litters (4 piglets per litter) were assigned randomly to 1 of the 2 groups on the basis of their litter origins (n = 12/group). Piglets in group 1 continued to be nursed by sows, whereas piglets in group 2 were weaned and housed in pens (2 piglets per pen) of the same animal facilities and had free access to a corn- and soybean meal-based diet and drinking water, as we previously described (10). Milk consumption by suckling piglets was measured with another similar group of 6 piglets over an 8-h period at d 21, 24, and 28 of age, using the weight-suckle-weight technique (14) and the mean value for the 3 measurements was used to represent nutrient intake by a piglet. An additional group of piglets was used for the measurement of milk consumption, because this technique was associated with stress on piglets. Feed intake by weanling piglets was determined during the 7-d period after weaning. On d 28, at 1 h after suckling or feeding, blood samples (~3 mL) were obtained from the jugular vein and piglets were then humanely killed after anesthesia, as we previously described (3). Plasma was obtained after centrifugation at 12,000 × g; 1 min and stored at −80°C. The whole small intestine was weighed and its length was measured after careful removal of luminal contents. The luminal contents were centrifuged at 12,000 × g; 1 min. The supernatant fluid was placed in liquid nitrogen and stored at −80°C until analysis for glutamine. In neonatal pigs, the small intestine was defined as the portion of the digestive tract between the pylorus and the ileocecal valve, with the first 10-cm segment being duodenum (13). The jejunum and ileum constituted ~40 and 60%, respectively, of the small intestine below the duodenum (13). A portion of mid-jejunum (~3 cm each in length) was placed in 4% paraformaldehyde for subsequent analysis of morphology (14) and another set of samples (~10 cm long) were obtained for glutathione analysis (15). Jejunal samples (~5 g) were placed in RNAlater solution (Ambion) and stored at −80°C before use for the isolation of total RNA.

Expt. 2 was conducted as for Expt. 1, except that 24 piglets were weaned and then assigned randomly to 1 of the 2 treatment groups (n = 12/group), representing supplementation with 1% L-glutamine (wt:wt) and 1.22% L-alanine (wt:wt; isonitrogenous control) to the corn- and soybean meal-based diet (10). The basal diet, which contained 21.0% crude protein (on an as-fed basis; 89.6% dry matter), was analyzed for amino acids using HPLC (1,7). The contents (percent of diet on an as-fed basis) of amino acids were as follows: arginine, 1.34; alanine, 1.27; aspartate+asparagine, 2.18; glutamate+glutamine, 3.36; glycine, 0.86; histidine, 0.56; isoleucine, 0.87; leucine, 1.73; lysine, 1.38; methionine, 0.35; phenylalanine, 0.97; proline, 1.52; serine, 0.76; threonine, 0.83; tryptophan, 0.24; tyrosine, 0.74; and valine, 0.97. The amount of supplemental glutamine was based on the results of our previous study that indicated its efficacy in preventing intestinal atrophy and enhancing growth in early-weaned piglets (10). At 28 d of age, 1 h after feeding, tissues were obtained, processed, and stored, as described in Expt. 1.

Alanine was chosen for isonitrogenous control from among nonessential amino acids on the basis of the following considerations. First, in contrast to glycine, alanine is not an antioxidant (16). Second, glycine, glutamate, aspartate, and metabolites of tyrosine (dopa and dopamine) are neurotransmitters (17); therefore, dietary supplementation of these amino acids may alter food intake by pigs. In addition, glutamate is a substrate for the synthesis of glutamine (10) and glutathione (16). Third, both glutamate and proline are major precursors for endogenous synthesis of arginine (5) and, thus, their addition to the diet may augment arginine provision in vivo (18). Fourth, in animals, serine and asparagine are readily converted to glycine and aspartate, respectively (4). Fifth, tyrosine metabolism via tyrosine hydroxylase requires tetrahydrobiopterin (17); therefore, its supplementation may reduce the availability of this essential cofactor for the generation of nitric oxide [a major vasodilator and a key regulator of smooth muscle relaxation (19)]. Sixth, cysteine is a sulfur-containing amino acid and a substrate for the synthesis of glutathione (an antioxidant) (16). The catabolism of increased amounts of cysteine leads to the production of H_2SO_4, which seriously disturbs acid-base balance in animals (17). Finally, all amino acids, except for leucine and lysine, are potential substrates for hepatic gluconeogenesis. However, alanine is not toxic and animals (including piglets) have a high capacity to catabolize this neutral amino acid (18). Thus, among nonessential amino acids, alanine is most appropriate for isonitrogenous control in this study. In our preliminary study, we found that adding 1.22% L-alanine (wt:wt) to the basal diet for 21- to 28-d-old weaned pigs did not affect food intake, small-intestinal weight and morphology, concentrations of glutamine and glutathione in jejunal mucosa, concentrations of glucose and arginine in plasma, whole-body weight gain, or expression of genes in the small intestine compared with no alanine supplementation (n = 6 pigs/treatment group; G. Wu, unpublished data).

Histological and biochemical analyses of the small intestine. Villus height, crypt depth, and lamina propria depth in jejunum were determined with the aid of a microscope (10× magnification), as we previously described (10,13). Free glutamine in the jejunal luminal content was analyzed using the HPLC method involving precolumn derivatization with *o*-phthaldialdehyde (1). Jejunal reduced glutathione (GSH)[8] and oxidized glutathione (GSSG) were measured using an HPLC method of Jones et al. (15), except that: *1*) fluorescence detection (Waters 2475 Multi λ Fluorescence Detector) was set at 590-nm excitation and 610-nm emission (0.0–7.5 min) to eliminate the appearance of amino acid peaks and at 335-nm excitation and 610-nm emission (7.5–38 min) for GSH and GSSG detection; and *2*) gain of the detection was set at 100 (0–32.2 min) for GSH detection and at 1000 (32.2–38 min) for GSSG detection. GSH and GSSG were quantified on the basis of authentic standards (Sigma Chemicals) using the Millennium-32 software and workstation.

RNA extraction. Frozen tissue (0.5 g) was homogenized in 5 mL TRIzol reagent (Invitrogen) and total RNA was isolated according to the manufacturer's recommendations. RNA integrity and quantity were analyzed using the Agilent 2100 Bioanalyzer and RNA 6000 Nano-LabChip kit (Agilent catalog no. 5065–4474). The 28S ribosome:18S ribosome peak areas ratio was ≥1.80 for all samples, indicating little degradation of RNA.

Generation of labeled antisense RNA for microarray. One microgram of total RNA was used as the starting material for amplification using Amino Allyl MessageAmp II aRNA Amplification kit (Ambion catalog no. 1753). The procedure consisted of reverse transcription with an oligo(dT) primer bearing a T7 promoter and arrayscript, a reverse transcriptase enzyme that catalyzes the synthesis of a full-length cDNA. The cDNA underwent second-strand synthesis and then used as template for in vitro transcription with T7 RNA polymerase and the incorporation of the modified nucleotide, 5-(3-aminoallyl)-UTP, into antisense RNA (aRNA). The aminoallyl-UTP contained a reactive primary amino group on the C5 position of uracil that was coupled to *N*-hydroxysuccinimidyl eater-derivatized reactive dyes (Cy3 and Cy5). In vitro

[8] Abbreviations used: aRNA, antisense RNA; GSH, reduced glutathione; GSSG, oxidized glutathione; ICAM-1, intercellular adhesion molecule-1; IL, interleukin; KLF-10, transforming growth factor β-inducible early growth response protein I krueppel-like factor 10; MAPK, mitogen-activated protein kinase; rRNA, ribosomal RNA.

transcription generated hundreds to thousands of aRNA copies of each mRNA in the sample. The quality and quantity of the amplified RNA were analyzed via capillary electrophoresis using RNA nano-assay technique (Agilent 2100 Bioanalyzer). No ribosomal RNA (rRNA) contamination was detected for all samples, indicating the high quality of the aRNA obtained.

Five micrograms of aRNA was used for the coupling reaction using the Mono-Reactive Cy3 and Cy5 dyes (Amersham Biosciences). In Expt. 1, jejunal RNA samples from each of 4 sow-reared piglets were used as controls and labeled with Cy3 (green), whereas RNA samples from each of 4 weaned piglets were labeled with Cy5 (red). In Expt. 2, jejunal RNA samples from alanine (control)- and glutamine-supplemented weaned piglets ($n = 4$/group) were labeled with Cy3 and Cy5, respectively. A dye swap was adopted for each experiment.

Microarray hybridization and analysis. One microgram of each labeled aRNA was used for hybridization to the porcine oligomicroarray. The paired aRNA samples were combined and fragmented using fragmentation reagents (catalog no. 8740, Ambion) and hybridized overnight with hybridization buffer (SlideHyb catalog no. 8861, Ambion) at 48°C to glass arrays with the Operon Pig Genome Oligo Set containing 11,000 oligonucleotides (genes spotted by the Microarray Core Facility, Department of Systems Biology and Translational Medicine, Texas A&M Health Science Center, Temple, TX). Following the hybridization, the arrays were washed and scanned using a GenePix 4000A scanner (Axon Instruments).

Real-time RT-PCR confirmation of gene expression. Real-time RT-PCR technology was employed to verify changes in the mRNA levels of select genes (**Supplemental Table 1**) obtained from the microarray analysis. First-strand cDNAs were synthesized from 1 μg of total RNA (10 ng for internal standard: 18S RNA) using oligo (deoxythymidine) primers, random hexamer primers, and SuperScript II Reverse Transcriptase, as we described (20). RT-PCR analysis was performed using the SYBR Green method and the ABI 7900 Sequence Detection System (Applied Biosystems). The thermal cycling parameters were as follows: 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Primers were designed using Primer Express Software version 1.5 (Applied Biosystems) (Supplemental Table 1). Values for cycle threshold, the value of cycle at which the fluorescence achieves a predetermined threshold, were determined using the Applied Biosystems Software. The cycle threshold values were analyzed using the generalized estimating equations model and the PROC GENMOD procedure of the Statistical Analysis System, as we described (21). All of the data were normalized with the 18S rRNA gene in the same samples and are expressed as the relative values to those of piglets fed the control diet.

Statistical analysis. Results are expressed as means ± SEM. Data on tissue metabolite concentrations and intestinal morphology were statistically analyzed using an unpaired t test. In microarray analysis, the acquired data were transformed (to accommodate the dye swap), normalized, and filtered using the GeneSpring v7.2 software package (Silicon Genetics, Agilent Technologies). Gene expression significance was assessed using multiple t tests and the Benjamini Hochberg false discovery rate multiple testing correction (20,21). P-values ≤ 0.05 were considered significant. The GeneSpring v7.2 software was used to categorize the genes that were up- and downregulated.

Results

Food intake, body weights, and small intestine weight. Food intake and daily body weight gain between 21 and 28 d of age were reduced ($P < 0.01$) by 36 and 47%, respectively, in weaned pigs compared with age-matched suckling piglets (Expt. 1; **Table 1**). At d 28, the small intestine weight was 26% lower ($P < 0.01$) in weaned than in sow-reared piglets (Table 1). Supplementing 1.0% L-glutamine to the diet for weaned piglets did not affect feed intake but increased ($P < 0.05$) daily body weight gain between 21 and 28 d of age by 19% compared with the control group (Expt. 2; Table 1).

Glutamine concentrations in plasma and jejunum. Glutamine concentrations in plasma and jejunum of 28-d-old pigs were affected ($P < 0.01$) by weaning and dietary glutamine supplementation (**Table 2**). Glutamine concentrations in jejunal lumen fluid, jejunal tissue, and plasma were 70, 38, and 30% lower ($P < 0.01$), respectively, in weaned than in sow-reared piglets (Expt. 1). Dietary glutamine supplementation increased ($P < 0.01$) concentrations of glutamine in jejunal lumen fluid, jejunal tissue, and plasma by 638, 107, and 46% ($P < 0.01$), respectively, compared with alanine-supplemented (control) piglets (Expt. 2).

Glutathione concentrations in jejunum. Concentrations of GSH in jejunal tissue of 28-d-old pigs were 25% lower ($P < 0.01$) in weaned than in sow-reared piglets (Expt. 1, Table 2). In contrast, weaning increased ($P < 0.01$) jejunal concentrations of GSSG. As a result, the GSSG:GSH ratio (an indicator of oxidative stress) was 59% greater ($P < 0.01$) in weaned than in sow-reared piglets. Dietary glutamine supplementation enhanced ($P < 0.01$) jejunal concentrations of GSH by 29% while reducing ($P < 0.01$) jejunal concentrations of GSSG by 18% and the GSSG:GSH ratio by 38% (Table 2).

Intestinal morphology and glutathione concentrations. Villus height, crypt depth, and lamina-propria depth in the jejunum of 21-d-old sow-reared piglets were 345 ± 14, 203 ± 12, and 205 ± 13 μm, respectively. Villus height in the jejunum was reduced ($P < 0.01$) by 43% in 28-d-old weaned piglets than in age-matched suckling piglets (Expt. 1, **Table 3**). Crypt depth or lamina propria depth did not differ between

TABLE 1 Food intakes and body weights of piglets[1]

Treatment	Dry matter intake d 21–28	Body weight d 21	Body weight d 28	Body weight gain d 21–28	Small intestine (d 28) Length	Small intestine (d 28) Weight
Expt. 1	*g/(kg body weight · d)*	*kg*	*kg*	*g/d*	*cm*	*g*
Suckling	33.5 ± 2.6	5.93 ± 0.34	7.98 ± 0.42	293 ± 17	714 ± 19	304 ± 8.5
Weaned	21.4 ± 2.3*	5.86 ± 0.37	6.94 ± 0.45*	154 ± 11*	733 ± 21	225 ± 6.3*
Expt. 2						
W+Ala	20.7 ± 2.5	5.95 ± 0.31	6.97 ± 0.44	146 ± 10	728 ± 16	229 ± 5.7
W+Gln	21.2 ± 2.8	5.91 ± 0.33	7.13 ± 0.48	174 ± 13†	717 ± 18	256 ± 6.1†

[1] Values are means ± SEM, $n = 6$ (feed intake) and 12 (other parameters). One gram dry matter contained 234 mg crude protein. *$P < 0.01$ vs. age-matched suckling piglets; †$P < 0.05$ vs. the W+Ala group. W+Ala, weaned pigs receiving dietary supplementation with isonitrogenous L-alanine (1.22%, wt:wt; control); W+Gln, weaned pigs receiving dietary supplementation with L-glutamine (1%, wt:wt).

TABLE 2 Concentrations of glutamine in plasma and jejunal lumen fluid, as well as GSH and GSSG in the jejunum of 28-d-old piglets[1]

Treatment	Glutamine		Jejunal tissue			
	In plasma	In jejunal lumen fluid	Glutamine	GSH	GSSG	GSSG/GSH
Expt. 1	*μmol/L*	*mmol/L*	*μmol/g tissue*		*nmol/g tissue*	*μmol/μmol*
Suckling	507 ± 42	3.76 ± 0.43	1.85 ± 0.11	2.56 ± 0.15	122 ± 6.0	0.049 ± 0.003
Weaned	354 ± 30*	1.12 ± 0.16*	1.14 ± 0.06*	1.93 ± 0.12*	148 ± 7.3*	0.078 ± 0.005*
Expt. 2						
W+Ala	371 ± 33	1.09 ± 0.13	1.27 ± 0.07	1.87 ± 0.10	153 ± 8.1	0.082 ± 0.005
W+Gln	543 ± 47*	8.04 ± 0.75*	2.63 ± 0.19*	2.42 ± 0.17*	126 ± 6.4*	0.051 ± 0.002†

[1] Values are means ± SEM, $n = 12$. *$P < 0.01$ vs. age-matched suckling piglets; †$P < 0.01$ vs. the W+Ala group. W+Ala, Weaned pigs receiving dietary supplementation with isonitrogenous L-alanine (1.22%, wt:wt; control); W+Gln, Weaned pigs receiving dietary supplementation with L-glutamine (1%, wt:wt).

weaned and sow-reared piglets. Dietary glutamine supplementation increased ($P < 0.01$) jejunal villus height by 38% but had no effect on crypt depth or lamina propria depth (Expt. 2, Table 3).

Effect of weaning on gene expression in the piglet small intestine. Compared with age-matched suckling piglets, mRNA levels for 18 genes were reduced ($P < 0.05$) by 35–77% in the jejunum of 28-d-old weaned piglets (**Table 4**). These downregulated genes were acyl-CoA dehydrogenase, *N*-acyl-D-glucosamine 2-epinerase, adenylate cyclase, ADP-ribosylation factor GTPase activating protein I, aminopeptidase A, apolipoprotein A-IV precursor, carnitine transporter 2, cathepsin F, DNA-binding protein inhibitor ID-2, fatty acid binding protein, insulin-like growth factor II precursor, leukocyte antigen-related protein, oxysterol binding protein-related protein 10, preprogalanin, sodium- and chloride-dependent creatine transporter I, somatostatin precursor, ubiquitin carboxyl-terminal hydrolase, and vanin-1.

There were 52–346% increases ($P < 0.05$; **Table 5**) in expression of 21 genes in the jejunum of weanling pigs compared with suckling piglets. The upregulated genes were aquaporin 8, core 2-β-16-*N*-acetylglucosaminyltransferase, cytochrome P450, galactoside 2-α-L-fucosyltransferase, glutathione transferase ω-1, diphosphomevalonate decarboxylase, hydroxymethylglutaryl-CoA synthase, 3-β-hydroxysteroid-δ^8- δ^7-isomerase, Ig α-chain C, Ig J-chain, Ig κ-chain C, Ig λ-chain, lysozyme, C-4 methyl sterol oxidase, nudix hydrolase-5, polymeric immunoglobulin receptor precursor, septin 5, and squalene epoxidase.

Effect of dietary glutamine supplementation on gene expression in the small intestine of weaned piglets. Intestinal expression of 8 genes decreased ($P < 0.05$) by 34–75% in weaned piglets supplemented with glutamine compared with alanine-supplemented (isonitrogenous control) weaned pigs (**Table 6**). These downregulated genes were casein kinase Iε, intercellular adhesion molecule-1 (ICAM-1) precursor, la protein homolog, mitogen-activated protein kinase-6 (MAPK-6), peptidyl-prolyl isomerase, Rho-related GTP-binding protein RhoE, pre-mRNA cleavage complex II protein, and transforming growth factor β-inducible early growth response

TABLE 3 Jejunal morphology in 28-d-old piglets[1]

Treatment	Villus height	Crypt depth	Lamina propria
Expt. 1		*μm*	
Suckling	468 ± 8.5	204 ± 8.1	205 ± 8.2
Weaned	265 ± 7.0*	223 ± 8.7	224 ± 8.8
Expt. 2			
W+Ala	269 ± 7.5	228 ± 7.9	229 ± 8.0
W+Gln	371 ± 9.2†	246 ± 7.7	248 ± 7.8

[1] Values are means ± SEM, $n = 12$. *$P < 0.01$ vs. age-matched suckling piglets; †$P < 0.01$ vs. the W+Ala group. W+Ala, Weaned pigs receiving dietary supplementation with isonitrogenous L-alanine (1.22%, wt:wt; control); W+Gln, Weaned pigs receiving dietary supplementation with L-glutamine (1%, wt:wt).

TABLE 4 Reduced expression of genes in the jejunum of 28-d-old weaned piglets compared with age-matched suckling piglets[1]

No.	Gene name	Common name	*P*-value	Change
				%
W1	NM_001608	Acyl-CoA dehydrogenase (long-chain specific) mitochondrial precursor)	0.049	−52
W2	NM_001114	Adenylate cyclase	0.003	−64
W3	NM_014570	ADP-ribosylation factor GTPase-activating protein 1	0.036	−65
W4	NM_001977	Aminopeptidase A	0.010	−41
W5	NM_000482	Apolipoprotein A-IV precursor	0.008	−52
W6	TC104654	Cathepsin F precursor	0.015	−35
W7	NM_002166	DNA-binding protein inhibitor ID-2	0.041	−77
W8	NM_000134	FZHUI fatty acid-binding protein intestinal	0.012	−50
W9	NM_000612	Insulin-like growth factor II precursor	0.027	−59
W10	NM_130440	Leukocyte antigen related protein precursor	0.047	−36
W11	NM_002910	*N*-Acyl-D-glucosamine 2-epimerase	0.046	−50
W12	NM_003060–2	Carnitine transporter 2	0.040	−46
W13	NM_017784	Oxysterol binding protein-related protein 10	0.012	−61
W14	NM_015973	Preprogalanin	0.019	−60
W15	NM_004654	Ubiquitin carboxyl-terminal hydrolase FAF-Y	0.038	−46
W16	NM_005629	Sodium- and chloride-dependent creatine transporter 1	0.002	−65
W17	NM_001048	Somatostatin precursor	0.028	−55
W18	NM_004666	Vanin-1	0.019	−76

[1] The sign (−) denotes a decrease in mRNA levels in the small intestine of weaned piglets compared with age-matched suckling piglets. FZHUI, fatty acid binding protein human intestine; FAF-Y, Y-linked fat facets protein related; CoA, coenzyme A; ID, inhibitor of DNA-binding protein.

TABLE 5 Enhanced expression of genes in the jejunum of 28-d-old weaned piglets compared with age-matched suckling piglets[1]

No.	Gene name	Common name	*P*-value	Change
				%
W19	NM_006579	3-β-Hydroxysteroid- δ^8-δ^7-isomerase (cholesterol δ-isomerase)	0.031	+55
W20	NM_001112683	Aquaporin 8	0.002	+220
W21	NM_006745	C-4 Methyl sterol oxidase	0.019	+76
W22	NM_006745–2	C-4 Methyl sterol oxidase	0.020	+147
W23	NM_004751	Core 2 β-16-N-acetylglucosaminyltransferase	0.009	+95
W24	NM_000786	Cytochrome P450 51A1 (sterol 14-α- demethylase; lanosterol 14-α-demethylase)	0.024	+51
W25	TC117335	Diphosphomevalonate decarboxylase	0.010	+54
W26	TC118958	Farnesyl diphosphate synthase	0.042	+102
W27	NM_000511	Galactoside 2-α-L-fucosyltransferase 2	0.033	+125
W28	NM_004832	Glutathione transferase ω-1 (glutathione-dependent dehydroascorbate reductase)	0.037	+206
W29	NM_002130	Hydroxymethylglutaryl-CoA synthase, cytoplasmic	0.019	+141
W30	TC104371	Ig α-chain C	0.024	+268
W31	TC119456	Ig κ-chain C region	0.033	+153
W32	TC103973	Ig λ-chain	0.049	+279
W33	NM_144646	Ig J-chain	0.021	+192
W34	NM_214392	Lysozyme	0.007	+346
W35	NM_014142	Nudix hydrolase-5	0.045	+52
W36	NM_004508	Plasminogen activator-inducible c54	0.031	+158
W37	NM_002644	Polymeric-immunoglobulin receptor precursor	0.018	+137
W38	TC104588	Septin 5 (peanut-like protein-1; cell division control related protein-1)	0.047	+153
W39	NM_003129	Squalene epoxidase	0.011	+67

[1] The sign (+) denotes an increase in mRNA levels in the small intestine of weaned piglets compared with age-matched suckling piglets.

protein I Krueppel-like factor 10 (KLF-10). In contrast, expression of 6 genes increased ($P < 0.05$) by 120–124% in the jejunum of weaned pigs in response to dietary glutamine supplementation (Table 6). These upregulated genes were AF-9 protein, endozepine, heme-binding protein, interleukin (IL)-13R-α-1, myosin, and signal recognition particle.

Real-time PCR confirmation of gene expression. Changes in mRNA levels for 12 genes were determined using real-time PCR analysis. The 18S rRNA gene was used as an internal standard (house-keeping gene). Results of the RT-PCR analysis indicated that compared with age-matched suckling piglets, mRNA levels for DNA-binding protein inhibitor ID-2 (−81%), insulin-like growth factor II precursor (−77%), ubiquitin carboxyl-terminal hydrolase Y-linked fat facets protein related (−92%), somatostatin precursor (−83%), and vanin (−87%) decreased ($P < 0.05$), whereas mRNA levels for aquaporin 8 (+315%), glutathione transferase ω-1 (+215%), hydroxymethylglutaryl-CoA synthase (cytoplasmic; +63%), and lysozyme

TABLE 6 Effects of dietary glutamine supplementation on gene expression in the jejunum of weaned piglets[1]

No.	Gene name	Common name	*P*-value	Change
				%
G1	NM_152221	Casein kinase I epsilon	0.046	−34
G2	TC105498	Intercellular adhesion molecule-1 precursor	0.032	−38
G3	NM_016648	La protein homolog (La ribonucleoprotein; La autoantigen homolog)	0.002	−41
G4	NM_002748–2	MAPK-6	0.018	−75
G5	NM_004792	Peptidyl-prolyl isomerase G (cyclophilin G)	0.003	−37
G6	NM_015885–3	Pre-mRNA cleavage complex II protein Pcf11	0.029	−61
G7	NM_005168	Rho-related GTP-binding protein RhoE (Rho8; Rnd3)	0.017	−59
G8	NM_005655	Transforming growth factor-β-inducible early growth response protein 1 (TGFB-inducible early growth response protein 1); (KLF 10)]	0.001	−64
G9	NM_004529–2	AF-9 protein	0.024	+121
G10	TC116763	Endozepine	0.035	+123
G11	NM_015987	Heme-binding protein	0.048	+124
G12	NM_001560	IL-13R-α1(IL-13R-α1; CD213A1 antigen)	0.034	+120
G13	NP276409	Myosin	0.016	+122
G14	NM_006947	Signal recognition particle 72K chain	0.023	+121

[1] The signs (−) and (+) denote decreased and increased mRNA levels, respectively, in the small intestine of weaned piglets receiving dietary supplementation with L-glutamine (1%, wt:wt), compared with piglets receiving dietary supplementation with isonitrogenous L-alanine (1.22%, wt:wt; control). La protein, a nuclear phosphoprotein first described as an autoantigen, with the name La deriving from the name of the patient in which the antibody was detected; AF-9, ALLI-fused gene from chromosome 9; CD, cluster designation; Pcf11, mammalian homolog cleavage and polyadenylation factor II subunit.

(+278%) increased ($P < 0.05$) in the jejunum of weaned piglets (Expt. 1). Additionally, supplementing glutamine to the diet for weanling piglets decreased ($P < 0.05$) mRNA levels for casein kinase I epsilon (−34%), MAPK-6 (−67%), and Krueppel-like factor 10 (KLF-10, −51%) compared with alanine-supplemented pigs (Expt. 2). The relative changes in gene expression revealed by RT-PCR analysis were similar to those indicated by the microarray analysis (Tables 4–6).

Discussion

After mammalian neonates are weaned from their mothers, they undergo tremendous changes in intestinal structure and function (8,9). However, the underlying molecular and cellular mechanisms are largely unknown. Due to the invasive nature of biochemical research on intestinal development, the piglet provides a useful animal model for studying the responses of the neonatal gut to weaning (10). With the recent availability of the microarray technology, which can analyze simultaneously expression of thousands of genes in a tissue (11), we identified significant changes in intestinal expression of key regulatory genes in response to weaning and dietary glutamine supplementation (Tables 4–6). Functionally, the glutamine treatment resulted in increased oxidative-defense capacity (Table 2), prevention of intestinal atrophy (Table 3), and enhanced growth performance (Table 1) of early-weaned piglets.

Weaning was associated with reduced expression of 18 genes out of 11,000 in the piglet small intestine (Table 4). Interestingly, these genes encode for proteins that are related to: *1*) the regulation of gene expression [DNA-binding protein inhibitor ID-2 (22)]; *2*) protein and peptide degradation [aminopeptidase A, cathepsin F, and ubiquitin carboxyl-terminal hydrolase (23)]; *3*) lipid metabolism [acyl-CoA dehydrogenase, carnitine transporter 2, and oxysterol binding protein-related protein 10 (24)]; *4*) signal transduction [adenylate cyclase and ADP-ribosylation factor GTPase-activating protein I (25)]; *5*) immune function [leukocyte antigen-related protein and vanin-1 (26)]; *6*) growth regulation (insulin-like growth factor II precursor and somatostatin precursor); *7*) aminosugar synthesis [*N*-acyl-D-glucosamine 2-epinerase (27)]; and *8*) intestinal transport [apolipoprotein A-IV precursor, fatty acid-binding protein, sodium- and chloride-dependent creatine transporter I, and preprogalanin (28)]. Downregulation of these genes is expected to reduce oxidative defense capacity, intestinal transport and utilization of dietary nutrients (particularly lipids and proteins), immune response, and synthesis of glycoproteins (major proteins secreted by the intestinal mucosa), as well as proliferation and differentiation of intestinal epithelial cells. This is consistent with the previous report that intestinal atrophy and dysfunction often occur in early-weaned piglets (8,9) in association with an increased GSSG:GSH ratio (Table 3), an indicator of cellular oxidative stress (16).

Expression of 21 genes was enhanced in the small intestine of piglets in response to early weaning (Table 5). These genes encode for proteins that play crucial roles in regulating: *1*) lipid metabolism [3-β-hydroxysteroid-Δ^8, Δ^7-isomerase, C-4 methyl sterol oxidase, diphosphomevalonate decarboxylase, hydroxymethylglutaryl-CoA synthase, and squalene epoxidase (29,30)]; *2*) water secretion [aquaporin-8 (31)]; *3*) aminosugar degradation (core 2-β-16-*N*-acetyl-glucosaminyltransferase); *4*) xenobiotic metabolism and oxidative defense [cytochrome P450, glutathione transferase ω-1 (32), and nudix hydrolase-5 (33)]; *5*) carbohydrate metabolism (galactoside 2-α-L-fucosyltransferase); *6*) immune function (Ig α chain C, Ig κ chain C, Ig λ chain, Ig J chain, lysozyme, polymeric Ig receptor precursor); and *7*) cell division [septin-5 (34)]. Emerging evidence shows that cAMP mediates expression of aquaporin-8 expression in small intestinal mucosa in response to cholera toxin (35). The enhanced expression of aquaporin-8, which stimulates water permeation across the gut (31), may help explain the increased secretion of water from intestinal mucosal cells and the malabsorption of water from the intestinal lumen and, therefore, diarrhea that frequently occurs in early-weaned piglets (36). Additionally, weaned piglets were fed a typical corn- and soybean meal-based diet that contained high levels of plant-origin antigens (37). Results from the present study indicate that a response to such a diet was increased expression of genes responsible for the synthesis of Ig in the small intestine (Table 4). Also, the increased expression of glutathione *S*-transferase [which catalyzes the conjugation of GSH to electrophilic substances (32)] and nudix hydrolase-5 [which eliminates toxic nucleotide derivatives from cells and regulates the levels of signaling nucleotides (33)] suggests the presence of oxidative stress in the small intestine of weaned piglets. In support of this view, the GSSG:GSH ratio in the jejunum was elevated by 59% in response to early weaning (Table 2).

Previous studies have shown that glutamine regulates MAPK activation and C-Jun signaling in enterocytes (38). In addition, this amino acid enhances intestinal oxidative metabolism, polyamine synthesis, ion transport, and cell proliferation (39,40) as well as cytoprotection via heat shock proteins (41,42). Consistent with these reports, we found that, in response to dietary glutamine supplementation, expression of 8 genes was downregulated in the small intestine (Table 6). These genes are related to cellular signaling transduction [casein kinase 1 epsilon and MAPK-6 (43)], the cell cycle [Rho-related GTP-binding protein (44)], apoptosis [KLF-10 (45)], immune activation (La antoantigen homolog and ICAM-1), protein modification [peptidyl-prolyl isomerase G (46)], and gene expression (pre-mRNA cleavage complex II). Enhanced expression of antigens and activation of leukocytes (e.g. lymphocytes and macrophages) in the intestinal epithelium because of activation of the MAPK-6 signaling may contribute to intestinal dysfunction and diarrhea in weanling pigs (36). In addition, peptidyl-prolyl isomerase G plays a role in posttranslational protein modifications and cell proliferation (46), which is likely unfavorable for intestinal integrity in weanling piglets. Likewise, ICAM-1 associates with receptors of the integrin family proteins, thereby playing an important role in immune activation (47). Also, Rho GTPase inhibits cell cycle progression by decreasing Ras (a signal transduction protein with an abbreviation that originated from rat sarcoma)- and Raf (an oncogene that encodes protein kinase with an abbreviation from rat fibrosarcoma)-induced fibroblast transformation (44). Furthermore, KLF-10 induces and promotes apoptosis through the mitochondrial apoptotic pathway (48). Therefore, downregulation of these genes provides an additional explanation for the effect of dietary glutamine supplementation on reducing intestinal damage and enhancing intestinal cell proliferation in early-weaned piglets (10).

Another beneficial effect of glutamine supplementation is increased expression of 6 genes (Table 6) related to transcription regulation [AF-9 protein (49)], lipid metabolism (endozepine), iron absorption (heme-binding protein), cytoskeletal structure and function (myosin), defense against pathological microorganisms [IL-13R-α-1 (50) and endozepine (51)], and regulation of nutrient metabolism (signal recognition particle 72K chain). AF-9 is a transcription factor that regulates gene expression and cell growth (52). IL-13 stimulates contractility of intestinal smooth muscle (50) and, thus, the movement of luminal digesta along the small intestine, which facilitates the digestion of macronutrients by various digestive enzymes and the absorption of resultant smaller molecules by enterocytes. Signal recognition particle (a cytoplasmic ribonucleoprotein), which consists of 1 RNA and 6 proteins, regulates gene expression and protein function (53). Additionally, endozepine, which is expressed in intestinal mucosal cells (54), has multiple functions in intestinal metabolism, physiology, and immunology. This polypeptide acts like acyl CoA-binding protein, therefore regulating lipid metabolism, assembly, and trafficking across the small intestine (55). Also, porcine endozepine has a potent antibacterial activity in the porcine gut (56). Because early weaning is often associated with immunological challenges in the intestine (36), elevated expression of endozepine in glutamine-supplemented piglets may protect the gut from infections during weaning. Collectively, these findings provide a mechanism for the effect of dietary glutamine supplementation on preventing intestinal atrophy and improving nutrient digestion and utilization in early-weaned piglets (10).

In conclusion, results of the microarray analysis reveal that early weaning resulted in increased expression of genes that promote oxidative stress and immune activation but decreased expression of genes related to nutrient utilization and cell proliferation in the piglet small intestine. In contrast, dietary supplementation of glutamine to weanling piglets enhanced expression of genes that prevent oxidative stress, improve antibacterial activity, enhance nutrient absorption, and stimulate cell growth. These novel findings reveal coordinate alterations of gene expression in response to weaning and aid in providing molecular mechanisms for explaining the previous observation that dietary glutamine supplementation prevents intestinal dysfunction and enhances the growth performance of early-weaned piglets. Further work is necessary to determine how these changes in gene expression translate into enhanced gut growth and function.

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Literature Cited

1. Wu G, Knabe DA. Free and protein-bound amino acids in sow's colostrums and milk. J Nutr. 1994;124:415–24.
2. Davis TA, Nguyen HV, Garciaa-Bravo R, Fiorotto ML, Jackson EM, Lewis DS, Lee DR, Reeds PJ. Amino acid composition of human milk is not unique. J Nutr. 1994;124:1126–32.
3. Reeds PJ, Burrin DG. The gut and amino acid homeostasis. Nutrition. 2000;16:666–8.
4. Wu G. Intestinal mucosal amino acid catabolism. J Nutr. 1998;128: 1249–52.
5. Wu G, Morris SM. Arginine metabolism: nitric oxide and beyond. Biochem J. 1998;336:1–17.
6. Flynn NE, Wu G. An important role for endogenous synthesis of arginine in maintaining arginine homeostasis in neonatal pigs. Am J Physiol Regul Integr Comp Physiol. 1996;271:R1149–55.
7. Wang X, Qiao SY, Yin YL, Yue LY, Wang ZY, Wu G. Deficiency or excess of dietary threonine reduces protein synthesis in jejunum and skeletal muscle of young pigs. J Nutr. 2007;137:1442–6.
8. Miller BG, James PS, Smith MW. Effect of weaning on the capacity of pig intestinal villi to digest and absorb nutrients. J Sci Food Agric. 1986;107:579–89.
9. Gu X, Li D, She R. Effect of weaning on small intestinal structure and function in the piglet. Arch Anim Nutr. 2002;56:275–86.
10. Wu G, Meier SA, Knabe DA. Dietary glutamine supplementation prevents jejunal atrophy in weaned pigs. J Nutr. 1996;126:2578–84.
11. Nguyen DV, Arpat AB, Wang N, Carroll RJ. DNA microarray experiments: biological and technological aspects. Biometrics. 2002;58:701–17.
12. Wu G, Bazer FW, Tuo W. Developmental changes of free amino acid concentrations in fetal fluids of pigs. J Nutr. 1995;125:2859–68.
13. Wang JJ, Chen LX, Li DF, Yin YL, Wang XQ, Li P, Dangott LJ, Hu WX, Wu G. Intrauterine growth restriction affects the proteomes of the small intestine, liver and skeletal muscle in newborn pigs. J Nutr. 2008;138: 60–6.
14. Wu G, Flynn NE, Knabe DA. Enhanced intestinal synthesis of polyamines from proline in cortisol-treated piglets. Am J Physiol Endocrinol Metab. 2000;279:E395–402.
15. Jones DP, Carlson JL, Samiec PS, Sternberg P, Mody VC, Reed RL, Brown LAS. Glutathione measurement is human plasma: evaluation of sample collection, storage and derivatization conditions for analysis of dansyl derivatives by HPLC. Clin Chim Acta. 1998;275:175–84.
16. Fang YZ, Yang G, Wu G. Free radicals, antioxidants, and nutrition. Nutrition. 2002;8:872–9.
17. Li P, Yin YL, Li D, Kim SW, Wu G. Amino acids and immune function. Br J Nutr. 2007;98:237–52.
18. Kim SW, Wu G. Dietary arginine supplementation enhances the growth of milk-fed young pigs. J Nutr. 2004;134:625–30.
19. Jobgen WS, Fried SK, Fu WJ, Meininger CJ, Wu G. Regulatory role for the arginine-nitric oxide pathway in metabolism of energy substrates. J Nutr Biochem. 2006;17:571–88.
20. Fu WJ, Hayne TE, Kohl R, Hu JB, Shi WJ, Spencer TE, Carroll RJ, Meininger CJ, Wu G. Dietary L-arginine supplementation reduces fat mass in Zucker diabetic fatty rats. J Nutr. 2005;135:714–21.
21. Fu WJ, Hu J, Spencer T, Carroll R, Wu G. Statistical models in assessing fold changes of gene expression in real-time RT-PCR experiments. Comput Biol Chem. 2006;30:21–6.
22. Kurooka H, Yokota Y. Nucleo-cytoplasmic shuttling of Id2, a negative regulator of basic helix-loop-helix transcription factors. J Biol Chem. 2005;280:4313–20.
23. Wilkinson KD. Regulation of ubiquitin-dependent processes by deubiquitinating enzymes. FASEB J. 1997;11:1245–56.
24. Lessmann E, Ngob M, Leitgesc M, Mingueta S, Ridgwayb ND, Hubera M. Oxysterol-binding protein-related protein (ORP) 9 is a PDK-2 substrate and regulates Akt phosphorylation. Cell Signal. 2007; 19:384–92.
25. Gillingham AK, Munro S. The small G proteins of the arf family and their regulators. Annu Rev Cell Dev Biol. 2007;23:579–611.
26. Berruyer C, Pouyet L, Millet V, Martin FM, LeGoffic A, Canonici A, Garcia S, Bagnis C, Naquet P, et al. Vanin-1 licenses inflammatory mediator production by gut epithelial cells and controls colitis by antagonizing peroxisome proliferators-activated receptor γ activity. J Exp Med. 2006;203:2817–27.
27. Maru I, Ohta Y, Murata K, Tsukada Y. Molecular cloning and identification of N-acyl-D-glucosamine 2-epimerase from porcine kidney as a rennin-binding protein. J Biol Chem. 1996;271:16294–9.
28. Anselmi L, Stella SL, Lakhter A, Hirano A, Tonini M, Sternini C. Galanin receptors in the rat gastrointestinal tract. Neuropeptides. 2005;39:349–52.
29. Cuthbert JA, Lipsky PE. Regulation of proliferation and Ras localization in transformed cells by products of mevalonate metabolism. Cancer Res. 1997;57:3498–505.
30. Shibata N, Arita M, Misaki Y, Dohmae N, Takio K, Ono T, Inoue K, Arai H. Supernatant protein factor, which stimulates the conversion of squalene to lanosterol, is a cytosolic squalene transfer protein and enhances cholesterol biosynthesis. Proc Natl Acad Sci USA. 2001; 98:2244–9.
31. Loo DDF, Wright EM, Zeuthen T. Water pumps. J Physiol. 2002;542: 53–60.
32. Wu GY, Fang YZ, Yang S, Lupton JR, Turner ND. Glutathione metabolism and its implications for health. J Nutr. 2004;134:489–92.
33. McLennan AG. The Nudix hydrolase superfamily. Cell Mol Life Sci. 2006;63:123–43.
34. Kinoshita M. The septins. Genome Biol. 2003;4:236.
35. Flach CF, Lange S, Jennische E, Lonnroth I. Cholera toxin induces expression of ion channels and carriers in rat small intestinal mucosa. FEBS Lett. 2004;561:122–6.
36. Ou DY, Li DF, Cao YH, Li XL, Yin JD, Qiao SY, Wu G. Dietary supplementation with zinc oxide decreases expression of the stem cell factor in the small intestine of weanling pigs. J Nutr Biochem. 2007;18: 820–6.
37. Li DF, Nelssen JL, Reddy PG, Blecha F, Hancock JD, Allee GL, Goodband RD, Klemm RD. Transient hypersensitivity to soybean-meal in the early-weaned pig. J Anim Sci. 1990;68:1790–9.
38. Rhoads M. Glutamine signaling in intestinal cells. JPEN J Parenter Enteral Nutr. 1999;23:S38–40.
39. Rhoads JM, Keku EO, Woodard JP, Bangdiwala SI, Lecce JG, Gatzy JT. L-Glutamine with D-glucose stimulates oxidative metabolism and NaCl absorption in piglet jejunum. Am J Physiol Gastrointest Liver Physiol. 1992;263:G960–6.
40. Rhoads JM, Argenzio RA, Chen WN, Rippe RA, Westwick JK, Cox AD, Berschneider HM, Brenner DA. L-Glutamine stimulates intestinal cell proliferation and activates mitogen-activated protein kinases. Am J Physiol Gastrointest Liver Physiol. 1997;272:G943–53.
41. Wischmeyer PE, Musch MW, Madonna MB, Thisted R, Chang EB. Glutamine protects intestinal epithelial cells: role of inducible HSP70. Am J Physiol Gastrointest Liver Physiol. 1997;272:G879–84.
42. Li N, Liboni K, Fang MZ, Samuelson D, Lewis P, Patel R, Neu J. Glutamine decreases lipopolysaccharide-induced intestinal inflammation in infant rats. Am J Physiol Gastrointest Liver Physiol. 2004;286: G914–21.
43. Fish KJ, Cegielska A, Getman ME, Landes GM, Virshup DM. Isolation and characterization of human casein kinase I epsilon (CKI), a novel member of the CKI gene family. J Biol Chem. 1995;270:14875–83.
44. Villalonga P, Guasch RM, Riento K, Ridley AJ. RhoE inhibits cell cycle progression and ras-Induced transformation. Mol Cell Biol. 2004;24: 7829–40.

45. Subramaniam M, Hawse JR, Johnsen SA, Spelsberg TC. Role of TIEG1 in biological processes and disease. J Cell Biochem. 2007;102: 539–48.
46. Lu KP, Hanes SD, Hunter T. A human peptidyl-prolyl isomerase essential for regulation of mitosis. Nature. 1996;380:544–7.
47. Luo BH, Carman CV, Springer TA. Structural basis of integrin regulation and signaling. Annu Rev Immunol. 2007;25:619–47.
48. Jin W, Di GH, Li JJ, Chen Y, Li WF, Wu J, Cheng TW, Yao M, Shao ZM. TIEG1 induces apoptosis through mitochondrial apoptotic pathway and promotes apoptosis induced by homoharringtonine and velcade. FEBS Lett. 2007;581:3826–32.
49. Fuse N, Yasumoto K, Takeda K, Amae S, Yoshizawa M, Udono T, Takahashi K, Tamai M, Tomita Y, et al. Molecular cloning of cDNA encoding a novel microphthalmia-associated transcription factor isoforms with a distinct amino-terminus. J Biochem. 1999;126: 1043–51.
50. Morimoto M, Morimoto M, Zhao AP, Madden KB, Dawson H, Finkelman FD, Mentink-Kane M, Urban JF, Wynn TA, et al. Functional importance of regional differences in localized gene expression of receptors for IL-13 in murine gut. J Immunol. 2006;176:491–5.
51. Pusch W, Jahner D, Spiess AN, Ivell R. Rat endozepine-like peptide (ELP): cDNA cloning, genomic organization and tissue-specific expression. Gene. 1999;235:51–7.
52. Fischer U, Heckel D, Michel A, Janka M, Hulsebos T, Meese E. Cloning of a novel transcription factor-like gene amplified in human glioma including astrocytoma grade I. Hum Mol Genet. 1997;6:1817–22.
53. Utz PJ, Hottelet M, Lei TM, Kim SJ. Geiger me, van Venrooij WJ, Anderson P. The 72-kDa component of signal recognition particle is cleaved during apoptosis. J Biol Chem. 1998;273:35362–70.
54. Steyaert H, Tonon MC, Tong YA, Smihrouet F, Testart J, Pellefier G, Vaudry H. Distribution and characterization of endogenous benzodiazepine receptor ligand (endozepine)-like peptides in the rat gastrointestinal tract. Endocrinology. 1991;129:2101–9.
55. Chang JL, Tsai HJ. Carp cDNA sequence encoding a putative diazepam-binding inhibitor/endozepine/acyl-CoA-binding protein. Biochim Biophys Acta. 1996;1298:9–11.
56. Agerberth B, Boman A, Andersson M, Jornvall H, Mutt V, Boman HG. Isolation of 3 antibacterial peptides from pig intestine: gastric-inhibitory polypeptide(7042), diazepam-binding inhibitor(32–86) and a novel factor, peptide-3910. Eur J Biochem. 1993;216:623–9.