Effect of supplemental L-threonine on mucin 2 gene expression and intestine mucosal immune and digestive enzymes activities of laying hens in environments with high temperature and humidity

M. M. M. Azzam,*† X. T. Zou,*[1] X. Y. Dong,* and P. Xie*

*Feed Science Institute, Zhejiang University, Hangzhou 310029, China;
and †Poultry Production Department, Mansoura University, Mansoura 35516, Egypt

ABSTRACT In addition to being an essential amino acid in protein synthesis, threonine is an integral component of gut function. To verify the effects of L-threonine on gut function, Babcock Brown layers (n = 960; 40 wk of age) were allocated to 5 dietary treatment groups, each of which included 6 replicates of 32 hens. Each group received the same basal diet formulated with corn, peanut meal, and crystalline amino acids. L-Threonine was added to the basal diet at 0 (control), 0.1, 0.2, 0.3, and 0.4% for 8 wk to acieve 0.47 (NRC), 0.57, 0.67, 0.77, and 0.87% threonine, respectively. Expressions of jejunal and ileal mucin 2 mRNA were increased linearly by increasing L-threonine ($P < 0.01$). At 0.4% L-threonine, the concentrations of IgA antibody in the mucosa of the ileum increased linearly ($P < 0.01$). No differences attributable to treatment were found among groups in the activity of digestive enzymes in the jejunum or ileum. It was concluded that dietary threonine requirements as reported in current NRC recommendations are insufficient for modern commercial laying hens raised in summer climates. The results suggest that threonine might function as a nutrient immunomodulator in maintaining intestinal barrier function.

Key words: laying hen, high temperature and humidity environmental climate, mucin 2 gene expression, intestine mucosal immune activity, digestive enzymes activity

2011 Poultry Science 90:2251–2256
doi:10.3382/ps.2011-01574

INTRODUCTION

Mucin 2 (**MUC2**) is secreted by goblet cells (Tytgat et al., 1996) and is a major component of the protective mucus layer, which acts as a biophysical layer protecting the underlying epithelium from damage and infection by pathogenic bacteria and acts as a substrate and fixing medium for commensal bacteria (Linden et al., 2008). The epithelial cell is more prone to be invaded by *Escherichia coli* when the mucus gel layer is improperly formed (Specian and Oliver, 1991; Lamont, 1992). Mucin 2 is a glycoprotein that is rich in threonine and proline residues (Strous and Dekker, 1992; Van Klinken et al., 1995).

The increased attention to avian mucosal immunity is important because it can aid in preventing colonization and eventually infection of mucosal epithelia. Proper nutrition may help in optimizing the gut function. Compared with other essential amino acids, threonine content in proteins of the intestine represents up to 30% (Neutra and Forstner, 1987), suggesting that threonine affects intestinal functionality and maintenance. In healthy rats, threonine restriction reduced small and large intestine mucin synthesis (Faure et al., 2005). Also, an increased supply of threonine, along with serine, proline, and cysteine, can improve mucin synthesis and reconstruction of commensal bacteria during colitis (Faure et al., 2006).

Moreover, threonine is involved with amylase secretion in the digestive tract. Block et al. (1966) showed that the threonine requirement for amylase synthesis is approximately 11% of the protein. Because digestive enzymes are made up of amino acids, if enough amino acids are provided, enzyme production will increase and enhance digestive function.

Studies of the response of the gastrointestinal system to dietary ingredients and additives may allow the nutritionist to determine the optimum ingredients and additives required to achieve maximum immune efficiency. No data exist in the literature regarding effects of an intake of dietary threonine in excess of current NRC (1994) recommendations on the gut function of laying hens in subtropical climates. A previous study in our laboratory showed that adding 0.2 and 0.3% L-threonine improved egg production and serum IgG,

Received April 26, 2011.
Accepted July 3, 2011.
[1]Corresponding author: xtzou@zju.edu.cn

respectively, and beyond 0.1% L-threonine the level of serum free threonine increased significantly (Azzam et al., 2011). An important question now is whether the current NRC (1994) threonine recommendation is sufficient for optimal gut function. The goal of this study was to answer that question.

MATERIALS AND METHODS

Birds and Housing

A total of 960 commercial laying hens (Babcock Brown) were divided into 5 groups of 192 birds each, which consisted of 6 replications of 32 birds each. They were arranged using a completely randomized design. The hens were housed 4 birds per cage under the same managerial conditions in a windowed poultry house. The photoperiod was 16L:8D throughout the experiment. The present study was carried out from June to August, which are considered to be severely hot and humid months in the south of China. The temperature and humidity inside the barn were recorded twice daily (at 0800 and 1400 h). The average ambient RH inside the barn was 85 ± 3% and the mean daily temperature was 30 ± 5°C. This study lasted 9 wk and included a 1-wk acclimation period and an 8-wk experimental period.

Diets

Hens were fed the same basal diet based on corn, peanut meal, and crystalline amino acids. Feed in mash form and water were provided freely. Crystalline L-threonine (98.5% Thr, PT Cheil Jedang, Indonesia) was added to the basal diet at 0 (control), 0.1, 0.2, 0.3, and 0.4%. Dietary treatments were achieved by the addition of crystalline L-threonine at the expense of inert filler to give 0.47 [current NRC (1994) requirement], 0.57, 0.67, 0.77, and 0.87% threonine.

Chemical Analysis of Diets

Ingredient composition and analyzed nutrients are presented in Tables 1 and 2, respectively. To determine amino acids, samples were hydrolyzed with 6 *M* HCl at 110°C for 24 h, and the major amino acid composition of hydrolysates was analyzed by HPLC (Hitachi L-8900 Amino Acid Analyzer, Tokyo, Japan).

Digestive Enzyme Estimation

The mucosa was scraped from the jejunum and ileum of 6 hens per treatment (1 hen/replicate); these mucosa as well as the pancreas were homogenized in ice-cold 0.1 *M* sodium chloride solution. The homogenates were then centrifuged at 20,000 × *g* for 30 min at 4°C and the supernatant was collected and stored at −70°C for enzyme assays. After thawing the homogenates and adjusting to room temperature, the activities of α-amylase (EC 3.2.1.1) and trypsin (EC 3.4.21.4) were analyzed by a microplate reader (SpectraMax M5, Molecular Devices, Sunnyvale, CA) using diagnostic kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the instructions of the manufacturer. The protein concentrations were determined using the Coomassie Brilliant Blue G-250 reagent with BSA as a standard, and enzyme activities were expressed as units per gram of protein.

Determination of Total IgA and IgG Antibody Production in the Intestine

From 6 additional hens per treatment, both the jejunum and ileum were separated and all connective tissue and fat were removed. These tissues were stored at −70°C until they were prepared for analysis. At the time of analysis, the jejunal samples were thawed at room temperature, 2 g of jejunal sample was weighed, and 20 mL of deionized water was added, and it was homogenized for 30 s with a mechanical homogenizer (Proc Scientific Inc., Oxford, CT). An aliquot (5 mL) of the sample was centrifuged at 20,000 × *g* for 30 min

Table 1. Composition and nutrient content of control diet

Item (% unless noted)	Value
Ingredient	
Yellow corn	60.2
Soybean meal (48%)	4.00
Peanut meal (47.8%)	19.6
Soybean oil	4.00
Dicalcium phosphate	1.85
Limestone (38% Ca)	8.00
Premix[1]	0.50
Salt	0.30
DL-Methionine	0.13
L-Lysine-HCl	0.12
L-Isoleucine	0.18
L-Tryptophan	0.03
L-Valine	0.09
L-Threonine[2]	0.00
Filler[3]	1.00
Nutrient level[4]	
CP	16.40 (16.56)
ME (kcal/kg)	2,902
Methionine	0.33 (0.31)
Lysine	0.73 (0.70)
Isoleucine	0.67 (0.68)
Tryptophan	0.19
Valine	0.72 (0.70)
Threonine	0.47 (0.48)
Calcium	3.61
Available phosphorus	0.47

[1]Premix provided the following per kilogram: vitamin A, 7,000 IU; vitamin D_3, 2,500 IU; vitamin E, 36 mg; vitamin K, 32 mg; vitamin B_1, 2 mg; vitamin B_2, 5.6 mg; vitamin B_6, 4 mg; vitamin B_{12}, 0.025 mg; nicotinic acid, 38 mg; folic acid, 1.1 mg; calcium pantothenate, 10 mg; biotin, 0.16 mg; Cu, 10 mg; Fe, 80 mg; Mn, 100 mg; Zn, 60 mg; I, 0.55 mg; Se, 0.12 mg.

[2]The diets were formulated to contain 0.47, 0.57, 0.67, 0.77, and 0.87% threonine.

[3]The dose titrations were achieved by addition of L-threonine at the expense of washed builder's sand.

[4]Values were calculated from data provided by Feed Database in China (2009). Analyzed levels are in parentheses.

Table 2. Analyzed concentrations of amino acids in the experimental diets[1]

	L-Threonine[2] (%)				
Item	0.0	0.1	0.2	0.3	0.4
Formulated dietary threonine	0.47	0.57	0.67	0.77	0.87
Essential amino acid					
Arginine	1.29	1.41	1.36	1.30	1.30
Histidine	0.34	0.36	0.36	0.34	0.36
Isoleucine	0.68	0.68	0.66	0.68	0.68
Leucine	1.22	1.31	1.29	1.24	1.24
Lysine	0.70	0.71	0.71	0.70	0.70
Methionine	0.31	0.32	0.32	0.32	0.31
Phenylalanine	0.74	0.81	0.80	0.79	0.81
Threonine	0.48	0.58	0.66	0.74	0.85
Valine	0.70	0.72	0.71	0.71	0.72
Nonessential amino acid					
Alanine	0.77	0.80	0.75	0.78	0.79
Aspartic acid	1.53	1.58	1.64	1.57	1.59
Glutamic acid	3.20	3.32	3.43	3.25	3.34
Glycine	0.73	0.76	0.79	0.74	0.76
Proline	0.75	0.80	0.80	0.78	0.78
Serine	0.73	0.76	0.78	0.75	0.78
Tyrosine	0.49	0.51	0.50	0.50	0.52

[1]Chemical composition data are the results of a chemical analysis conducted in duplicate.

[2]Crystalline L-threonine (98.5% Thr, PT. Cheil Jedang, Indonesia).

at 4°C. The supernatant was obtained and stored at −20°C for antibody analyses. Antibodies in jejunum and ileum were analyzed by a microplate reader (SpectraMax M5, Molecular Devices) using a sandwich ELISA using chicken-specific IgA and IgG ELISA quantitation kits (R&D Systems, Minneapolis, MN), respectively, according to the instructions of the manufacturer, and absorbance was measured at 450 nm. The concentrations of IgA and IgG were determined using standard curves constructed from the standards run on the plate and were expressed as micrograms per milliliter.

MUC2 mRNA Expression Assay

Total RNA was isolated from chick tissues (~100 mg; ileum and jejunum) using TRIzol procedure (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The quality of total RNA was checked by both native RNA electrophoresis on 1.0% agarose gel in 1× TAE buffer (Tris 2.0 *M*, acetic acid 1.0 *M*, and EDTA 0.1 *M*, pH 8.0) and the UV absorbance ratio at 260 and 280 nm. The complementary DNA was synthesized from 2 μg of total RNA by Moloney-murine leukemia virus reverse transcriptase (Takara, Dalian, China) at 42°C for 60 min with oligo dT-adaptor primer following the protocol of the manufacturer. The abundance of mucin (MUC2) was determined on a StepOne Plus Real-Time PCR system (ABI 7500, Applied Biosystems, Foster City, CA).

The primers for MUC2 were as follows: 5′-TCACCCTGCATGGATACTTGCTCA-3′, forward; 5′-TGTCCATCTGCCTGAATGACAGGT-3′, reverse. The primer for GAPDH was as follows: 5′-GCCATCACAGCCACACAGA-3′, forward; 5′-TTTCCCCACAGCCTTAGCA-3′, reverse. Primer PCR reaction used SYBR PrimeScript RT-PCR kit (Takara). The PCR program was 95°C for 30 s, followed by 42 cycles of 95°C for 3 s, 60°C for 10 s, and 72°C for 30 s. The standard curve was determined using pooled samples. Each sample was performed in duplicate and no template control was included. Specificity of the amplification was verified at the end of the PCR run using melting curve analysis. The relative expression quantity was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The value represents an n-fold difference relative to the calibrator.

Statistical Analyses

Data were statistically analyzed by 1-way ANOVA by SPSS 16.0 for Windows (SPSS Inc., Chicago, IL). When significant differences were found ($P < 0.05$), Turkey posthoc tests were performed. The effect of supplemental levels of L-threonine was determined using orthogonal polynomials for linear, quadratic, and cubic effects.

RESULTS AND DISCUSSION

To our knowledge, no other reports exist concerning the evaluation of L-threonine on gut function in subtropical summer climates. In the current study, expression of jejunal and ileal MUC2 mRNA was increased linearly ($P < 0.01$) by increasing levels of L-threonine (Figures 1 and 2). Proteins and specific amino acids have been found to change mucin secretion and may interact directly with goblet cells or with the enteric nervous system to elicit an alteration in mucin production (Montagne et al., 2000; Claustre et al., 2002; Faure et al., 2005). Threonine represents approximately 11% of the amino acids in the human MUC2 protein (Gum, 1992). Montagne et al. (2004) reported that the

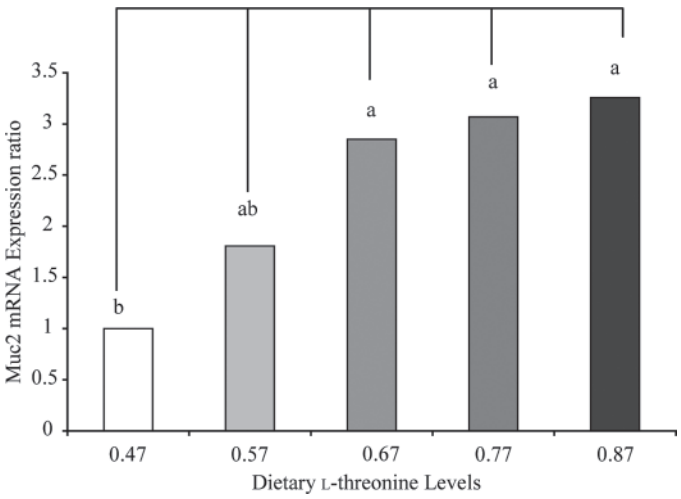

Figure 1. Effect of L-threonine on jejunal mucin 2 (MUC2) mRNA expression of laying hens in environments with high temperature and humidity. Changes in MUC2 mRNA expression are normalized to glyceraldehyde 3-phosphate dehydrogenase mRNA and expressed relative to the control. Values are means ± SEM (n = 6 hens/treatment). Means on each bar with no common letter differ significantly at $P < 0.01$.

hydroxyl group of threonine and serine is necessary for ester linkages on the mucin amino acids backbone to carbohydrate groups that make up the majority (50–80%) of the molecular weight of mucin. The alterations in mucin production could affect thickness, viscosity, and integrity of the mucus layer. Such alterations in the mucus layer have implications for gut protective functions and may affect intestinal nutrient absorption (Horn et al., 2009). Our result is in agreement with the result of Horn et al. (2009) who found that MUC2 mRNA abundance increased significantly in ducks with

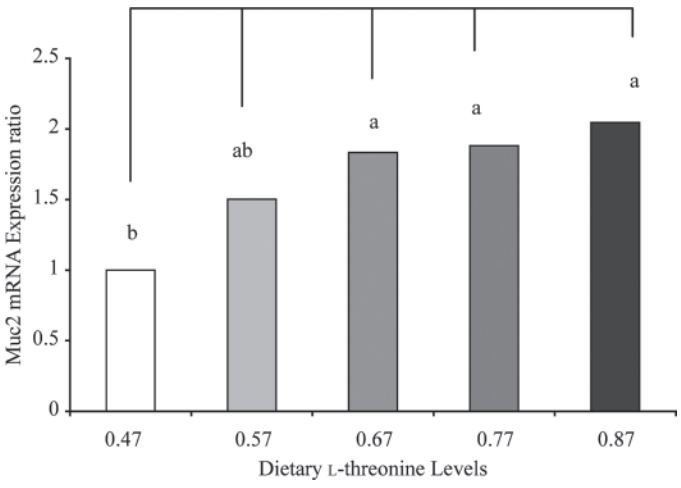

Figure 2. Effect of L-threonine on ileal mucin 2 (MUC2) mRNA expression of laying hens in environments with high temperature and humidity. Changes in MUC2 mRNA expression are normalized to glyceraldehyde 3-phosphate dehydrogenase mRNA and expressed relative to the control. Values are means ± SEM (n = 6 hens/treatment). Means on each bar with no common letter differ significantly at $P < 0.01$.

Table 3. Effect of graded levels of L-threonine on intestine mucosal immunity of laying hens in subtropical summer climates

	Jejunum (μg/mL)		Ileum (μg/mL)	
Item	IgA	IgG	IgA	IgG
Threonine (%)				
0.0	147.82	11.82	137.71[b]	11.05
0.1	149.58	12.94	138.07[b]	12.61
0.2	157.28	14.04	140.19[b]	12.53
0.3	159.98	14.04	140.84[ab]	12.49
0.4	148.36	13.83	148.98[a]	12.69
SEM	5.9	0.91	2.55	0.71
Effect				
Linear	0.41	0.05	0.001	0.07
Quadratic	0.07	0.22	0.06	0.18
Cubic	0.16	0.62	0.34	0.26

[a,b]Means within a row with no common superscripts differ significantly ($P < 0.05$).

increasing dietary threonine after 14 d of feeding. In contrast, Chee et al. (2010) and Horn et al., (2009) found that MUC2 mRNA expressions were not affected by dietary threonine restriction or supplementation in broilers. It has been reported that the gut function was changed by many factors of intestinal stress. For example, the exposure of broilers to 30°C for 24 h results in the alteration of intestinal bacterial flora and the intestinal epithelial structure (Burkholder et al., 2008). In inflammatory bowel diseases such as ulcerative colitis and Crohn's disease the mucus layer and mucin synthesis were impaired (Corfield et al., 2000). Moreover, mucin production is enhanced during intestinal inflammation (Deplancke and Gaskins, 2001). For example, Rémond et al. (2009) showed that ileitis increased gastrointestinal threonine uptake and mucin production. A previous study in our laboratory showed that adding 0.2 and 0.3% L-threonine improved egg production and serum IgG, respectively (unpublished data). Also, L-threonine supplementation above 0.1% significantly increased the level of serum free threonine. Intestinal mucin has many functions, such as lubrication and protection from pathogens, detoxifying heavy metal binding, maximizing the immune system by the accumulation of secretory IgA, acting as a diffusion barrier for nutrients and macromolecules, and protecting the underlying epithelial cells (Forstner and Forstner, 1994).

At 0.4% L-threonine the concentrations of IgA antibody in the ileum increased linearly ($P < 0.01$; Table 3). This suggests that threonine may have a direct effect on immunity in the intestine. The B cells activated in mucosal-associated lymphoid tissues differentiate to plasma cells, which home to the lamina propria and produce secretory IgA. The effector function of secretory IgA is to prevent the penetration of microorganisms by blocking the adherence to mucous membranes, improve bactericidal function, and neutralize bacterial toxins (Walker, 1976; Mestecky and McGhee, 1987; Bai et al., 2000). No difference attributable to threonine supplementation was found on the concentration of IgG antibody in the ileum or the jejunum. However, the ad-

Table 4. Effect of graded levels of L-threonine on enzyme activities (U/g of protein) in the pancreas and mucosal homogenates of the jejunum and ileum of laying hens in subtropical summer climates[1]

	Jejunum		Ileum		Pancreas	
Item	Trp	AMS	Trp	AMS	Trp	AMS
Threonine (%)						
0.0	5.89	166.65	97.88	0.90	402.05	1.32
0.1	6.13	177.61	102.36	0.91	414.28	1.58
0.2	10.71	177.54	105.21	1.00	462.95	1.56
0.3	6.62	173.86	105.12	0.96	432.25	1.50
0.4	6.06	176.03	98.17	0.98	416.11	1.50
SEM	2.44	22.90	7.40	0.10	26.55	0.25
Effect						
Linear	0.88	0.77	0.84	0.38	0.44	0.61
Quadratic	0.12	0.73	0.20	0.68	0.06	0.40
Cubic	0.88	0.74	0.75	0.93	0.71	0.55

[1]AMS = α-amylase activity.

dition of L-threonine at 0.3% in the diet resulted in increasing levels of IgG ($P = 0.05$). In the current study, L-threonine supplementation modulated the concentration of IgA mainly in the ileum rather than the jejunum because the ileum may be the preferred region for bacterial colonization. However, the jejunum is recognized as the major site for nutrient digestion (Iji et al., 2001) and absorption. In current study, ileum IgA has a specific requirement for threonine that is much higher than is required for egg production or serum IgG production (unpublished data). This means that very high threonine is required for gut function. Recently, Dahiya et al. (2007) also found that *Lactobacillus* populations were significantly higher in the ceca of birds receiving 0.8% methionine than in those of birds given diets with 0, 0.2, and 0.4%.

No differences attributable to treatment were found among groups in the activity of digestive enzymes (Table 4). However, trypsin activity tended to increase quadratically ($P = 0.06$) at 0.2% L-threonine in the pancreas. Our result is in agreement with the results of Kadam et al. (2008) who found that although injection of threonine in ovo did not influence digestive enzyme activities in the proventriculus, jejunum, and pancreas at 21 d posthatch, a tendency existed for levels to be higher in the threonine-injected bird.

In conclusion, dietary threonine requirements as reported in NRC (1994) are insufficient for modern commercial laying hen strains reared in subtropical summer climates to maximize the gut function, and threonine might be a nutrient immunomodulator that affects intestinal barrier function. It is necessary to know whether L-threonine supplementation, along with low-protein diets and other essential amino acids (lysine and methionine), can give the same trend during the same conditions.

ACKNOWLEDGMENTS

The research was supported by the earmarked fund for Modern Agro-Industry Technology Research System (Beijing, China).

REFERENCES

Azzam, M., X. Y. Dong, P. Xie, C. Wang, and X. T. Zou. 2011. The effect of supplemental L-threonine on laying performance, serum free amino acids, and immune function of laying hens under high-temperature and high-humidity environmental climates. J. Appl. Poult. Res. 20:361–370.

Bai, X., X. Liu, and Y. Su. 2000. Inhibitory effects of intestinal mucus on bacterial adherence to cultured intestinal epithelial cells after surface burns. Chin. Med. J. (Engl.) 113:449–450.

Block, R. J., K. W. Weiss, and D. B. Cornett. 1966. The amino acid composition of proteins. Pages 149–295 in The Amino Acid Composition of Proteins and Foods. R. J. Block and D. Bolling, ed. Charles C. Thomas, Springfield, IL.

Burkholder, K. M., K. L. Thompson, M. E. Einstein, T. J. Applegate, and J. A. Patterson. 2008. Influence of stressors on normal intestinal microbiota, intestinal morphology, and susceptibility to *Salmonella* Enteritidis colonization in broilers. Poult. Sci. 87:1734–1741.

Chee, S. H., P. A. Iji, M. Choct, L. L. Mikkelsen, and A. Kocher. 2010. Functional interactions of manno-oligosaccharides with dietary threonine in chicken gastrointestinal tract. I. Growth performance and mucin dynamics. Br. Poult. Sci. 51:658–666.

Claustre, J., F. Toumi, A. Trompette, G. Jourdan, H. Guignard, J. A. Chayvialle, and P. Plaisancie. 2002. Effects of peptides derived from dietary proteins on mucus secretion in rat jejunum. Am. J. Physiol. 283:521–528.

Corfield, A. P., N. Myerscough, R. Longman, P. Sylvester, S. Arul, and M. Pignatelli. 2000. Mucins and mucosal protection in the gastrointestinal tract: New prospects for mucins in the pathology of gastrointestinal disease. Gut 47:589–594.

Dahiya, J. P., D. Hoehler, A. G. Van Kessel, and M. D. Drew. 2007. Effect of different dietary methionine sources on intestinal microbial populations in broiler chickens. Poult. Sci. 86:2358–2366.

Deplancke, B., and H. R. Gaskins. 2001. Microbial modulation of innate defence: Goblet cells and the intestinal mucus layer. Am. J. Clin. Nutr. 73:1131S–1141S.

Faure, M., C. Mettraux, D. Moennoz, J. P. Godin, J. Vuichoud, F. Rochat, D. Breuille, C. Obled, and I. Corthesy-Theulaz. 2006. Specific amino acids increase mucin synthesis and microbiota in dextran sodium-treated rats. J. Nutr. 136:1558–1564.

Faure, M., D. Moennoz, F. Montigon, C. Mettraux, D. Breuille, and O. Ballevre. 2005. Dietary threonine restriction specifically reduces intestinal mucin synthesis in rats. J. Nutr. 135:486–491.

Feed Database in China. 2009. Tables of feed com-position and nutritive values in China. Accessed May 2009. http://www.chinafeeddata.org.cn.

Forstner, G., and J. F. Forstner. 1994. Gastrointestinal mucus. Pages 1255–1283 in Physiology of the Gastrointestinal Tract. 3rd ed. R. Johnson and P. Leonard, ed. Raven Press, New York, NY.

Gum, J. R., Jr. 1992. Mucin genes and the proteins they encode: Structure, diversity, and regulation. Am. J. Respir. Cell Mol. Biol. 7:557–564.

Horn, N. L., S. S. Donkin, T. J. Applegate, and O. Adeola. 2009. Intestinal mucin dynamics: Response of broiler chicks and White Pekin ducklings to dietary threonine. Poult. Sci. 88:1906–1914.

Iji, P. A., A. Saki, and D. R. Tivey. 2001. Body and intestinal growth of broiler chickens on a commercial starter diet. 1. Intestinal weight and mucosal development. Br. Poult. Sci. 42:505–513.

Kadam, M. M., S. K. Bhanja, A. B. Mandal, R. Thakur, P. Vasan, A. Bhattacharyya, and J. S. Tyagi. 2008. Effect of in ovo threonine supplementation on early growth, immunological responses and digestive enzyme activities in broiler chickens. Br. Poult. Sci. 49:736–741.

Lamont, J. T. 1992. Mucus: The front line of intestinal mucosal defense. Ann. N. Y. Acad. Sci. 664:190–201.

Linden, S. K., P. Sutton, N. G. Karlsson, V. Korolik, and M. A. McGuckin. 2008. Mucins in the mucosal barrier to infection. Mucosal Immunol. 1:183–197.

Livak, K. J., and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25:402–408.

Mestecky, J., and J. R. McGhee. 1987. Immunoglobulin A (IgA): Molecular and cellular interactions involved in IgA biosynthesis and immune response. Adv. Immunol. 40:153–245.

Montagne, L., C. Piel, and J. P. Lalles. 2004. Effect of diet on mucin kinetics and composition: Nutrition and health implications. Nutr. Rev. 62:105–114.

Montagne, L., R. Toullec, and J. P. Lalles. 2000. Calf intestinal mucin: Isolation, partial characterization, and measurement in ileal digesta with an enzyme-linked immunosorbent assay. J. Dairy Sci. 83:507–517.

Neutra, M. R., and J. F. Forstner. 1987. Gastrointestinal mucus: Synthesis, secretion and function. Pages 975–1009 in Physiology of the Gastrointestinal Tract. 2nd ed. Raven Press, New York, NY.

NRC. 1994. Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press, Washington, DC.

Rémond, D., C. Buffiere, J.-P. Godin, P. P. Mirand, C. Obled, I. Papet, D. Dardevet, G. Williamson, D. Breuille, and M. Faure. 2009. Intestinal inflammation increases gastrointestinal threonine uptake and mucin synthesis in enterally fed minipigs. J. Nutr. 139:720–726.

Specian, R. D., and M. G. Oliver. 1991. Functional biology of intestinal goblet cells. Am. J. Physiol. 260:C183–C193.

Strous, G. J., and J. Dekker. 1992. Mucin-type glycoproteins. Crit. Rev. Biochem. Mol. Biol. 27:57–92.

Tytgat, K. M., F. J. Opdam, A. W. Einerhand, H. A. Büller, and J. Dekker. 1996. MUC2 is the prominent colonic mucin expressed in ulcerative colitis. Gut 38:554–563.

Van Klinken, B. J., J. Dekker, H. A. Büller, and A. W. Einerhand. 1995. Mucin gene structure and expression: Protection vs. adhesion. Am. J. Physiol. 269:G613–G627.

Walker, W. A. 1976. Host defense mechanisms in the gastrointestinal tract. Pediatrics 57:901–916.