Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

Maggot Antimicrobial Peptide Effect on TGF-β_4 and TNF-α mRNA Expression in Small Intestinal Mucosa from *Salmonella pullorum*-Infected Chickens

Wang ZT[§1], Wang JG[§2], Zhang YS[1], Zhang XL[1], Xi JF[1], Li CC[1], Huang CF[1] and Shen H[1*]

[1]College of Animal Science and Technology, Shihezi University, Xinjiang, 832003, PR China
[2]College of Agronomy, Shihezi University, Xinjiang, 832003, PR China
*Corresponding author: shenhong98@163.com

ARTICLE HISTORY (17-005)

Received: November 1, 2016
Revised: April 15, 2017
Accepted: April 19, 2017
Published online: May 18, 2017

Key words:
Chickens
Intestinal mucosa
Maggots
Salmonella
TGF-β_4
TNF-α

ABSTRACT

To explore the effects of maggot antimicrobial peptide treatment of *Salmonella pullorum*-infected chickens on the mRNA expression levels of TGF-β_4 and TNF-α in small intestinal mucosa, the duodenum, jejunum and ileum mucosa were isolated 3, 5 and 7 days after induced AMP, non-induced AMP, antibiotics or maggot treatment and the TGF-β_4 and TNF-α mRNA expression was evaluated by quantitative real-time PCR. We found that mRNA of TGF-β_4 was highly expressed in the duodenum, jejunum, and ileum when *Salmonella pullorum*-infected chickens were treated with antibiotics, non-induced AMP, and AMP, respectively ($P<0.001$). Similarly, TNF-α mRNA was highly expressed in the duodenum and jejunum when *Salmonella pullorum*-infected chickens were treated with induced AMP and maggots, respectively ($P<0.001$), and the expression of TNF-α mRNA was higher in response to the use of induced AMP than antibiotics on day 5 after treatment ($P<0.05$). Therefore, maggot AMP functional efficiency, along with evaluation of TGF-β_4 and TNF-α produced by small intestinal mucosa, may be useful for the prevention and treatment of *Salmonella pullorum* when developing new chicken feed additives.

To Cite This Article: Wang ZT, Wang JG, Zhang YS, Zhang XL, Xi JF, Li CC, Huang CF and Shen H, 2017. Maggot antimicrobial peptide effect on TGF-β_4 and TNF-α mRNA expression in small intestinal mucosa from *Salmonella pullorum*-infected chickens. Pak Vet J, 37(3): 281-286.

INTRODUCTION

Pullorum disease (PD) is a bacterial disease caused by *Salmonella pullorum* through both horizontal and vertical transmission that primarily affects 2-3 week old chicks (Gong *et al*., 2013). The disease is extremely difficult to eradicate in vivo, and can exist for a long time. Salmonellosis is one of the most common endemic diseases in China, being responsible for large economic losses to the poultry industry. Currently, antibiotics are still an effective measure in addition to slaughter and vaccines in the treatment of *Salmonella pullorum* infection (Barrow *et al*., 2011). However, the widespread use of antibiotics is causing *Salmonella*-resistance to increase (Wang *et al*., 2012). Consequently, substitutes for antibiotics are urgently required for use in poultry production. Antimicrobial peptides (AMPs) have attracted increasing attention in recent years because they do not easily lead to drug resistance. These compounds are polypeptides produced by organisms to protect the host against infection (Garcia *et al*., 2013). To date, millions of AMP has been isolated from animals, plants, and insects. Maggots are housefly larvae. They live in harsh environments are surrounded by pathogenic bacteria. They are not susceptible to disease because of AMPs in their body. The antibacterial mechanism of AMPs is different from that of antibiotics; therefore, it is difficult for organisms to form drug resistance to these compounds. It is well known that AMP can partially depolarize the bacterial cytoplasmic membrane, which subsequently interferes with electron transport, leading to cell death (Padhi *et al*., 2014). Therefore, maggot AMPs would have broad prospects of application as new substitute antibiotics.

The effects of infection of chickens by *Salmonella pullorum*, which belong to *Salmonella enteric*, first manifest in the intestines (Priyantha *et al*., 2012). The intestinal mucosa immune cells can protect biological organisms from invasion by various pathogens in the lumen (Liang *et al*., 2016). Immunity of chickens can be divided into innate and acquired responses, which include

[§]These authors contributed equally to this work

cell-mediated immunity and antibody-mediated immunity (Carter *et al*., 2009). Cell-mediated immune response occurs via T cells and is mediated by cytokines and chemical signals. More than 20 kinds of cytokines have been reported in the intestinal genes of chickens (DeVries *et al*., 2006), including transforming growth factor-β_4 (TGF-β_4) and tumor necrosis factor-α (TNF-α). It has been reported that TGF-β_4 exists in vivo in chickens. Furthermore, chicken TGF-β_4 is equivalent to mammalian TGF-β_1 in terms of function and effect (Chowdhury *et al*., 2003). TNF-α plays a central role in intestinal inflammation disease as a pro-inflammatory cytokine (Kaser *et al*., 2010). Chicken homologs of TGF-β_4 and TNF-α have been cloned and sequenced.

In this study, chickens were artificially infected with *Salmonella pullorum* following treatment with maggot AMP, antibiotics and maggots after symptom onset. The small intestinal mucosa mRNA was then isolated at 3, 5, and 7 days after infection. qRT-PCR was subsequently used to measure the small intestinal mucosa of mRNA expression of TGF-β_4 and TNF-α, after which the values were compared between groups.

MATERIALS AND METHODS

Ethics statement: This study was approved by the Ethical Committee of Animal Experiments of the Animal Science and Technology College, Shihezi University.

Bacteria preparation: *Salmonella pullorum* (CVCC 578) were cultured in Luria Bertani (LB) liquid nutrient medium at 37°C until the logarithmic growth phase was attained. The bacterial cells were collected by centrifugation (tabletop centrifuge, 8000 g, 5 min), after which the cell concentrations were adjusted to 1×10^7 CFU/mL.

Experimental chickens: Induction of maggots, preparation of maggot AMPs and antibacterial activity assays of the AMPs were conducted as previously described (Wang *et al*., 2017).

Specific pathogen free (SPF) *Avian* male newly-hatched chicks were purchased from a local hatchery. All chicks were raised in cages while provided with feed and drinking water *ad libitum*. When they were 14-days-old, the chicks in four groups were injected with 2 mL of *S. pullorum* suspension (1×10^7 CFU/mL) into the pectoralis. The fifth group (referred to as the healthy group) was not injected with *Salmonella pullorum*. All chicks presented with pullorosis diarrhea symptoms at 24 h post injection. A total of 200 chicks of the same size and weight that showed pullorosis symptoms were then divided into four groups (maggots; non-induced *Salmonella pullorum* AMP; induced *Salmonella pullorum* AMP; antibiotics) at random. Chicks then received the appropriate treatments with crude extracts of AMP, maggots or antibiotics for the next 7 d. The chicks in induced *S. pullorum* AMP groups and non-induced *Salmonella pullorum* AMP groups were given 3 mL of the crude AMP extract (1 mg AMP/mL) daily. Antibiotics (*Gentamycin sulfate*) were added into the drinking water at 100 mg/L. The AMP content of live maggots was 0.5 μg AMP/g fresh weight. These four treatments continued for 3-5 d until the disease symptoms disappeared.

Small intestine mucosa collection: Chicks were slaughtered 3, 5, and 7 d after the above treatments were started and their intestinal tracts were dissected. Four cm long sections of the duodenum, jejunum and ileum were excised. Samples were flash frozen in liquid nitrogen and stored at -80°C until RNA purification.

RNA isolation and Reverse transcription: The total RNA from small intestine mucosa was isolated using an RNAeasy mini kit (TIANGEN Co., Beijing, China) according to the manufacturer's instructions, after which it was eluted with 30 μl RNase-free water. The purity and concentration of RNA were determined based on the absorbance at 260 nm (Nano Drop ND1000). A total of 1 μg of total RNA was immediately reverse transcribed using a Prime Script RT Reagent Kit (TaKaRa Co., Dalian, China) according to the manufacturer's instructions (Pan *et al*., 2012).

Real-time quantitative RT-PCR: The PCR primers used for RT-PCR are listed in Table 1. The PCR mixture consisted of 2 μl cDNA, 10 μl SYBR Green I Master Mix (Roche Co., Shanghai, China), 0.4 μl of each forward and reverse primer (100 μM) and 7.2 μl of RNase-free water to give a final volume of 20 μl. Amplification and detection of the specific products were performed using a LightCycler 480II Real-Time PCR System (Roche Co. Ltd., Shanghai, China) with the following program: one cycle of 95°C for 5 min, followed by 40 cycles of 95°C for 15 s, 57°C for 30 s, and 72°C for 30 s, and final a melting curve was determined. Each PCR reaction sample was subjected to real-time PCR in triplicate.

Statistical analysis: The resulting threshold cycle values were normalized against the endogenous control (β-actin), and the fold changes in expression of the target genes were determined using the equation $2^{\wedge-\Delta\Delta Ct}$ (Livak *et al*., 2001). Fold change values generated by different lines were analyzed by one-way ANOVA using SPSS version 17.0 (IBM, Armonk, NY). A $P<0.05$ was considered to indicate significance.

RESULTS

RNA isolation and cDNA: RNA was successfully isolated from small intestine mucosa of all five groups chickens used in present study. All three strips of RNA were clear upon gel electrophoresis. These results suggest that RNA quality was good and undegraded during the extraction process. The OD_{260nm}/OD_{280nm} values were between 1.8 and 2.1 for all samples based on ultramicro nucleic acid analysis, indicating that the samples were suitable for follow-up experiments.

TGF-β_4 mRNA expression: The TGF-β_4 mRNA expression was higher after antibiotics treatment relative to the control, with especially significant differences being observed on day 3 ($P<0.001$; Fig. 1 A). The TGF-β_4 expression levels in the jejunum showed that the non-induced AMP group was significantly upregulated relative to the other three groups (Fig. 1 B). The level of TGF-β_4 mRNA in the induced AMP group and the non-induced AMP group were significantly higher than those in the

antibiotics group (P<0.001; Fig. 1C). However, when compared with the antibiotics group, the mRNA expression of TGF-β_4 in the induced AMP group and the non-induced AMP group increased obviously at day 5 (P<0.001; Fig. 1B and C). These results showed that maggot AMP could induce the secretion of TGF-β_4 in the jejunum and ileum mucosa, while this factor was secreted in the duodenum following stimulation by antibiotics.

The expression levels of TGF-β_4 mRNA were high in the duodenum of the antibiotics group on day 3, 5, and 7 (P<0.001; Fig. 2). In the other groups, lower expression of TGF-β_4 mRNA was observed on day 3 (Fig. 2A). Regardless of the treatment time point, the TGF-β_4 expression levels were low in the ileum in all treatment groups, although they were somewhat higher in the AMP group than the antibiotics group. When compared with other groups, the expression of TGF-β_4 from the non-induced AMP group was highest in the jejunum (Fig. 2). Based on the above results, the induced AMP (P<0.05) and non-induced AMP (P<0.001) facilitated the expression of TGF-β_4 mRNA in the jejunum mucosa up to day 5, after that it decreased (Fig. 2B).

Overall, these results indicate that: (1) mRNA of TGF-β_4 was highly expressed in the duodenum in response to application of antibiotics to *S. pullorum*-infected chickens; (2) mRNA of TGF-β_4 was highly expressed in the jejunum in response to the use of non-induced AMP to treat *S. pullorum*-infected chickens; and (3) mRNA of TGF-β_4 was highly expressed in the ileum in response to AMP treatment of *S. pullorum*-infected chickens.

TNF-α mRNA expression: The mRNA expression levels of TNF-α from three groups were higher than those of the antibiotics group in the duodenum (Fig. 3A). There was a significant difference in the level of TNF-α mRNA expression between the induced AMP group and the antibiotics group (P<0.001) (Fig. 3A). The levels of TNF-α expression in the duodenum and jejunum from the maggots group were higher than those of the antibiotics group and non-induced AMP group at 3, 5, and 7 days (Fig. 3A and Fig. 3B). When compared to the antibiotics group, the induced AMP group and non-induced AMP group had higher expression levels of TNF-α on day 3 and 5 (Fig. 3C). Overall, the induced AMP group and the antibiotics group showed significant differences from the duodenum to the ileum at day 5 (P<0.001) (Fig. 3). These findings indicate that induced AMP can enhance TNF-α secretion in the small intestinal mucosa on day 5 after treatment compared to antibiotics.

There was a significant difference in TNF-α mRNA expression in the jejunum between the maggot group and the antibiotics group (P<0.001). TNF-α mRNA expression in the duodenum and ileum from the induced AMP group and the non-induced AMP group were higher than in the antibiotics group at 3 and 5 days (Fig. 4A and Fig. 4B). When compared with the antibiotics group, the levels of TNF-α expression in the duodenum and jejunum from the induced AMP group and the non-induced AMP group differed significantly on day 7 (P<0.05; Fig. 4C). In general, these data show that induced AMP and non-induced AMP enhanced TNF-α mRNA expression in the duodenum mucosa on day 3, 5, and 7 post-treatment.

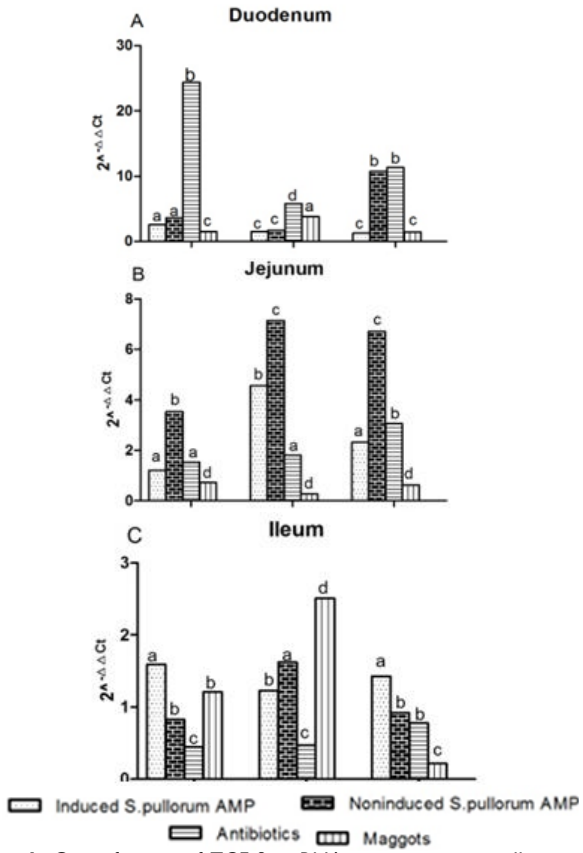

Fig. 1: Quantification of TGF-β_4 mRNA expression in small intestine mucosa isolated from recovered chickens on duodenum, jejunum and ileum. Data at each time point are the average of $2^{-\Delta\Delta Ct}$. Means with different superscripts are significantly different.

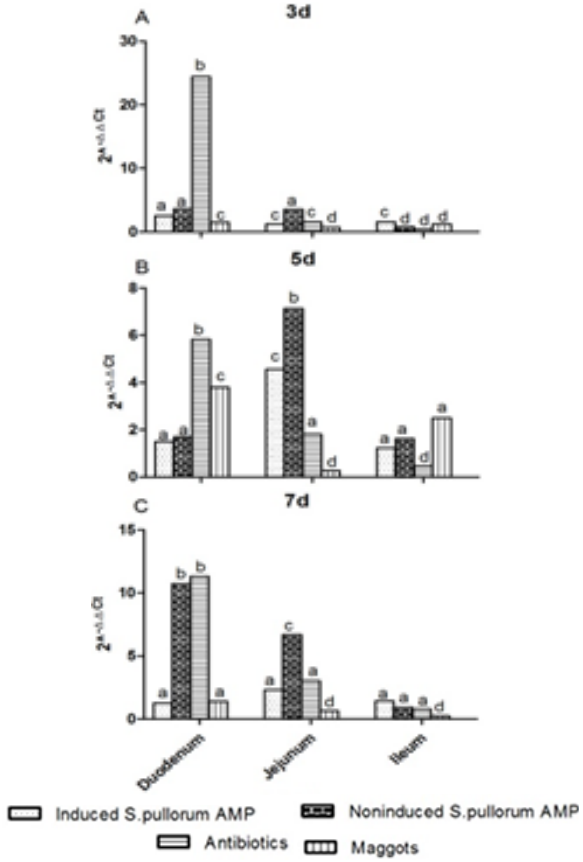

Fig. 2: Quantification of TGF-β_4 mRNA expression in small intestine mucosa isolated from recovered chickens on day 3, 5, and 7. Data at each time point are the average of $2^{-\Delta\Delta Ct}$. Means with different superscripts are significantly different.

Table 1: PCR primers used for qRT-PCR

Gene	Primer sequence	Fragment size (bp)	Reference
TGF-β_4	For: GGGGTCTTCAAGCTGAGCGT Rev: TTGGCAATGCTCTGCATGTC	240	Kramer *et al.* (2003)
TNF-α	For: TGAGTTGCCCTTCCTGT Rev: CAGAGCATCAACGCAAA	497	Malek *et al.* (2004)
β-action	For: GTGATGAAGCCCAGAGCAAAAGAG Rev: AGGGTGCTCCTCAGGGGCTACT	143	Xiao *et al.* (2014)

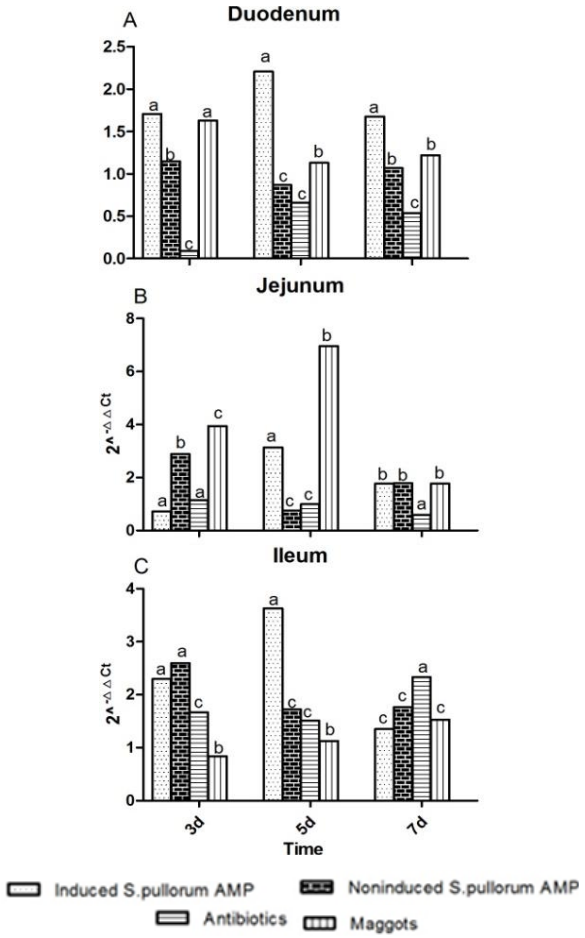

Fig. 3: Quantification of TNF-α mRNA expression in small intestine mucosa isolated from recovered chickens on duodenum, jejunum and ileum. Data at each time point are the average of $2^{-\Delta\Delta Ct}$. Means with different superscripts are significantly different.

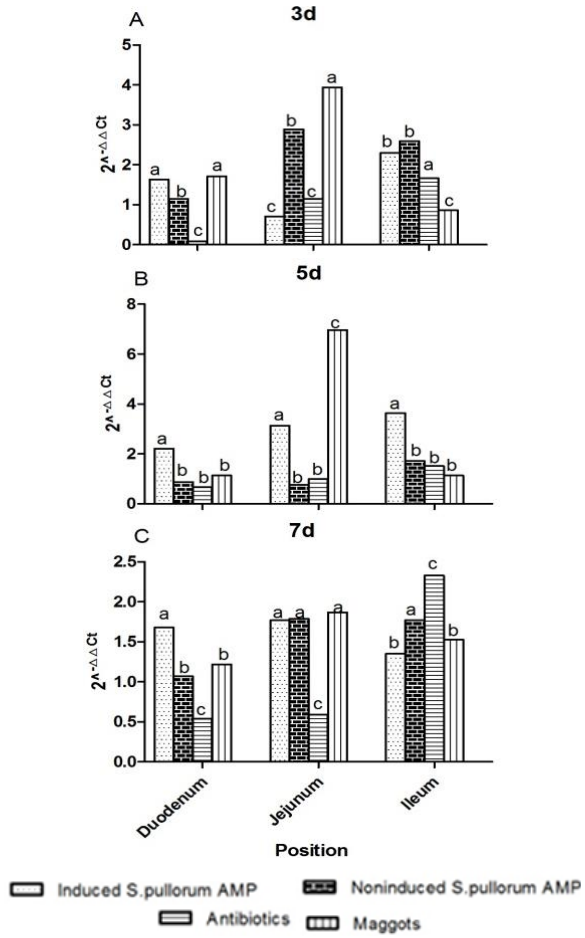

Fig. 4: Quantification of TNF-α mRNA expression in small intestine mucosa isolated from recovered chickens on day 3, 5, and 7. Data at each time point are the average of $2^{-\Delta\Delta Ct}$. Means with different superscripts are significantly different.

Based on the above results, the following conclusions can be drawn: (1) the level of TNF-α mRNA was highly expressed in the duodenum by the use of induced AMP to heal the *S. pullorum*-infected chickens; (2) the level of TNF-α mRNA was highly expressed in the jejunum by the use of maggots to treat *S. pullorum*-infected chickens, and (3) the mRNA expression levels of TNF-α were higher following treatment with induced AMP than antibiotics to heal the *S. pullorum*-infected chickens on day 5 after treatment.

DISCUSSION

Salmonella pullorum is a serious disease that impacts poultry throughout the world, resulting in economic losses to the poultry industry. Chickens are the natural host of *S. pullorum*, and antibiotics are still the main method to control and treat pullorum disease (Barrow *et al*., 2011; Huang *et al.*, 2016). With the extensive use of antibiotics, bacterial resistance is becoming increasingly prominent; therefore, in this study we attempted to use maggot AMP to treat pullorum disease. The results revealed that maggot AMP could also be used to treat *S. pullorum*-infected chickens. These results were identical to those of previous studies of *S. pullorum*-infected chickens (Zhou *et al*., 2014). Despite the advanced nature of AMP treatment of pullorum disease, there are still risks of side effects; accordingly, future studies investigating the differential cykotine mRNA expression effects of small intestinal mucosa are warranted.

Cytokines are essential effector molecules of the immune response to PD in infected chickens (Swaggerty *et al*., 2006). A study by Chowdhury *et al*. (2003) revealed the presence of TGF-β_4, which is only exists in vivo in chickens. In this study, we explored expression levels of TGF-β_4 and TNF-α mRNA in the small intestinal mucosa from *Salmonella pullorum*-infected chickens that had been treated using different methods. *S. pullorum*-infected chickens had been treated using different methods showed varying mRNA expression levels of

TGF-β_4. The changes of the expression of TGF-β_4 mRNA may be caused by *Salmonella pullorum*-infected chicks were treated using different methods. Pro-inflammatory cytokines (TGF-β_4) suppressed the anti-inflammatory response to resistance SE in chickens (Swaggerty *et al*., 2014). TGF-β4 is produced by T cells that were transformed from CD4+Th17 cells (Cao *et al*., 2012). In addition, while antibiotics primarily led to up-regulated TGF-β_4 mRNA expression in the duodenum, maggot AMP led to increased levels in the jejunum and ileum. The maggot AMP may promote TGF-β_4 expression, which in turn increases secretion by mucosa in the jejunum and ileum. The expression of TGF-β_4 was increase in *Salmonella enteritidis* infected chicks at early phase of infection (Karaffová *et al*., 2015). One study showed that the mRNA expression levels of TGF-β_4 were up-regulated in the spleens of 6-week-old *S. pullorum*-infected chickens (Beal *et al*., 2004), which was similar to maggot AMPs were treated with 15-day-old *S. pullorum*-infected chicks in the intestinal mucosal. However, unlike the present study, the mRNA expression levels of TGF-β_4 was a difference in *Salmonella enteria*-infected chickens peripheral blood mononuclear cells (Pan *et al*., 2012). This was likely due to the organs investigated secrete different cytokines. It is also possible than the types of chickens evaluated differed between studies.

TNF-α plays a central role in intestinal inflammation disease and is the therefore the target of treatment for this affliction (Perey *et al*., 2015). Indeed, inhibition of the activity of TNF-α has been shown to effectively reduce intestinal inflammation (Hollander *et al*., 2013). Probiotic could induce the sensitization of the host by increasing TNF-α (Hua, *et al*., 2010). Huang *et al*. (2008) report that *Bacillus* induced TNF-α in mesenteric lymph nodes of mice. In the present study, we found that the mRNA expression levels of TNF-α isolated from the duodenum and jejunum mucosa of chickens treated by induced AMP therapy were higher than those of animals that received antibiotics therapy. In contrast to the present study, Dong *et al*. (2016) have shown that dietary *Enterococcus fecalis* increasing the expression levels of TNF-α in jejunum and ileum mucosa. Moreover, our results indicated that TNF-α mRNA expression was up-regulated in response to treatment with *S. pullorum*-infected chickens after day 5 by using the induced AMP, and that the levels were significantly higher than those observed in response to antibiotic therapy. These results are similar to previous studies that chickens fed with *Clostridium butyricum* probiotic had higher concentration of jejunum mucosa TNF-α than those in colistin sulfate antibiotic treatment at 3 and 7 days (Zhang *et al*., 2016). To the best of our knowledge, this is the first study of maggot AMP effect on TNF-α mRNA expression in the small intestinal mucosa of *Salmonella pullorum*-infected chickens. Although the results of this study provide insight into alternative methods for the treatment of infected chickens, further research is needed.

Acknowledgments: This study was supported by the Specialized Research Fund for the National Natural Science Foundation of China (Grant No.31660703). The authors have no competing interests.

Authors contribution: Wang ZT executed the experiment and contributed in preparation of the manuscript. Wang JG took care of the laboratory animal. Shen H conceived and designed the study. Zhang YS, Xi JF and Li CC collected the tissue samples. Zhang XL and Huang CF analyzed the data. All authors approved the final manuscript.

REFERENCES

Barrow PA and Freitas-Neto OC, 2011. Pullorum disease and fowl typhoid-New thoughts on old diseases: A review. Avian Pathol 40:1-13.

Beal PK, Powers C, Wigley P, *et al*., 2004. Temporal dynamics of the cellular, humoral and cytokine responses in chickens during primary and secondary infection with Salmonella enterica serovar Typhimurium. Avian Pathol 33:25-33.

Cao AT, Yao S, Gong B, *et al*., 2012. Th17 cells upregulate polymeric Ig receptor and intestinal IgA and contribute to intestinal homeostasis. J Immunol 189:4666-73.

Carter AJ, Adams MR, Woodward MJ *et al*., 2009. Control strategies for Salmonella colonisation of poultry: the probiotic perspective. Food Sci Tech Bull Funct Foods 5:103-15.

Chowdhury VS, Nishibori M and Yoshimura Y, 2003. Changes in the expression of TGF β-isoforms in the anterior pituitary during withdrawal and resumption of feeding in hens. Gen Comp Endocrinol 133:1-7.

DeVries ME, Kelvin AA, Xu L, *et al*., 2006. Defining the origins and evolution of the chemokine/chemokine receptor system. J Immunol 176:401-15.

Dong ZL, Wang YW, Song D, *et al*., 2016. The effects of dietary supplementation of pre-microencapsulated Enterococcus fecalis and the extract of Camellia oleifera seed on growth performance, intestinal morphology, and intestinal mucosal immune functions in broiler chickens. Anim Feed Sci Tech 212:42-51.

Garcia F, Villegas E, Espino-Solis GP, *et al*., 2013. Antimicrobial peptides from arachnid venoms and their microbicidal activity in the presence of commercial antibiotics. J Antibiot 66:3-10.

Gong JS, Xu M and Zhu CH, 2013. Antimicrobial resistance, presence of integrons and biofilm formation of Salmonella pullorum isolates from eastern China. Avian Pathol 42:290-4.

Hollander D, 2013. Intestinal Permeability Barrier in Crohn's Disease: The Difficulty in Shifting the Paradigm. Dig Dis Sci 58:1827-9.

Hua MC, Lin TY, Lai MW, *et al*., 2010. Probiotic Bio-Three induces Th1 and anti-inflammatory effects in PBMC and dendritic cells. World J Gastroenterol 16:3529-40.

Huang JM, La RM, Nunez A, *et al*., 2008. Immunostimulatory activity of Bacillus spores. FEMS Immunol Med Microbiol 53:195-203.

Huang YS, Wu YC, Hu CW, *et al*., 2016. Isolation and characterization of salmonella spp. in sheltered wild birds in Taiwan. Pak Vet J 36:472-6.

Kaser A, Lee AH, Franke JN, *et al*., 2010. E09 ER stress, paneth cells and intestinal inflammation. J Crohns Colitis 4:8-9.

Karaffová V, Bobíková K, Husáková, *et al*., 2015. Interaction of TGF-β4 and IL-17 with IgA secretion in the intestine of chickens fed with E. faecium AL41 and challenged with S Enteritidis. Res Vet Sci 100:75-9.

Kramer J, Malek M and Lamont SJ, 2003. Association of twelve candidate gene polymorphisms and response to challenge with *Salmonella enteritidis* in poultry. Anim Genet 34:339-48.

Liang M, Ruo YP, Lei Z, *et al*., 2016. Invasion by Trichinella spiralis infective larvae affects the levels of inflammatory cytokines in intestinal epithelial cells in vitro. Exp Parasitol 170:220-6.

Livak KJ and Schmittgen TD, 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25:402-8.

Malek M, Hasenstein JR and Lamont SJ, 2004. Analysis of chicken TLR4, CD28, MIF, MD-2, and LITAF genes in a *Salmonella enteritidis* resource population. Poult Sci 83:544-9.

Padhi A, Sengupta M, Sengupta S, *et al*., 2014. Antimicrobial peptides and proteins in mycobacterial therapy: current status and future prospects. Tuberculosis 94:363-73.

Pan ZM, Fang Q, Geng SZ, *et al*., 2012. Analysis of immune-related gene expression in chicken peripheral blood mononuclear cells following *Salmonella enterica* serovar Enteritidis infection in vitro. Res Vet Sci 93:716-20.

Perey AC, Weishaar IM and McGee DW, 2015. The effect of ROCK on TNF-α-induced CXCL8 secretion by intestinal epithelial cell lines is mediated through MKK4 and JNK signaling. Cell Immunol 293: 80-6.

Priyantha MAR, Vipulasiri AA and Gunawardana, 2012. Salmonella control in poultry breeder farms in Sri Lanka: Effects of oral antibiotic treatment on whole blood agglutination test with Salmonella pullorum antigen. Int J Livest Prod 3:21-4.

Swaggerty CL, Kaiser P, Rothwell L, *et al*., 2006. Heterophil cytokine mRNA profiles from genetically distinct lines of chickens with differential heterophil-mediated innate immune responses. Avian Pathol 35:102-8.

Swaggerty CL, Pevzner IY and Kogut MH, 2014. Selection for pro-inflammatory mediators yields chickens with increased resistance against Salmonella enterica serovar Enteritidis. Poult Sci 93:535-44.

Wang LC, Zhang TT and Zhou YM, 2012. Protective effects of zinc-bearing clinoptilolite on broilers challenged with Salmonella pullorum. Poult Sci 91:1838-45.

Wang ZT, Wang JG, Zhang YS, *et al*., 2017. Antimicrobial peptides in housefly larvae (*Musca domestica*) affect intestinal *Lactobacillus acidophilus* and mucosal epithelial cells in *Salmonella* pullorum-infected chickens. Kafkas Univ Vet Fak Derg 23:423-30.

Xiao JH, WU YP, He YY, *et al*., 2014. Differential expression of *Pep T1* mRNA in small intestine of Chongren Spotty chicken. Acta Vet ET Zootech Sin 45:191-6.

Zhang L, Zhang L, Zhan XA, *et al*., 2016. Effects of dietary supplementation of probiotic, Clostridium butyricum, on growth performance, immune response, intestinal barrier function, and digestive enzyme activity in broiler chickens challenged with Escherichia coli K88. Anim Sci Biotechnol 7:1-9.

Zhou G, Wang JG, Zhu XQ, *et al*., 2014. Induction of maggot antimicrobial peptides and treatment effect in Salmonella pullorum-infected chickens. J Appl Poult Res 23:376-83.