DEVELOPMENTAL GENE EXPRESSION OF HOST DEFENSE PEPTIDES IN IMMUNE ORGANS AND THE SMALL INTESTINE OF TURKEY POULTS *(MELEAGRIS GALLOPAVO)*

By

Shaimaa Kamal Hamad

Thesis submitted to the Faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Masters of Science

In

Animal and Poultry Sciences

Rami A. Dalloul, Chair
Eric A. Wong, Co-chair
Michael E. Persia

September 1, 2016
Blacksburg, VA

DEVELOPMENTAL GENE EXPRESSION OF HOST DEFENSE PEPTIDES IN IMMUNE ORGANS AND THE SMALL INTESTINE OF TURKEY POULTS (MELEAGRIS GALLOPAVO)

Shaimaa Kamal Hamad

ABSTRACT

Host defense peptides (HDPs) are a large group of small positively charged peptides that play an important role in innate immunity. Their role is more critical at early ages when other components of the immune system have not fully developed. There are three classes of avian HDPs: avian beta defensins (AvBDs), cathelicidins (Cath) and liver-expressed antimicrobial peptide 2 (LEAP-2). The objective was to compare expression of HDPs in male turkey poults at day of hatch (D0), D7, D14, D21 and D28 from the thymus, spleen, bursa, duodenum, jejunum and ileum. The expression of AvBD1, AvBD2, AvBD8, AvBD9, AvBD10, AvBD13, Cath2, Cath3 and LEAP-2 was measured using qPCR (n=6 birds/tissue/age). Data were analyzed by one-way ANOVA and Tukey's test, and significance considered at $P \leq 0.05$. AvBDs and Caths exhibited greater expression in immune organs than intestinal tissues, with the greatest expression of AvBDs observed in the spleen. The intestinal tissues showed very low expression of AvBDs except for AvBD10 at D0. Similar to AvBDs, Caths expression in the immune organs was greater than the intestinal tissues with the spleen having the greatest expression among immune organs. Conversely, LEAP-2 showed greater expression in the intestinal tissues than in the immune tissues, which showed very low LEAP-2 expression unlike other HDPs. Understanding the differential expression of HDPs could reveal the innate immune status of poults, and may subsequently allow improvement of their health through appropriate mitigation strategies.

Key words: HDPs, turkeys, innate immunity, avian beta-defensins, LEAP-2

DEDICATION

I would like to dedicate this thesis to the soul of my great father, my great mother, my academic father Dr. Farid Stino and my priceless husband Ahmed El-Omda.

Thank you my dad for supporting me until the last minute of your life. Thank you my mother, Dr. Stino and my husband for your support and prayers.

ACKNOWLEDGEMENTS

Dr. Rami Dalloul. Thank you very much for everything. First of all, thanks for accepting me into your lab and giving me this great opportunity. Thanks for pushing me to work hard and teaching me all that I need to improve in my career. Thanks for helping me in the hardest days of my life. I will never forget all that you did for me and I could never thank you enough. I could not have dreamt of a better advisor.

Dr. Eric Wong. Thank you very much for being my co-advisor. Thanks for your help and your ever patience. Thanks for being here all the time to reply to my many questions. I could not have finished this Masters without you. I do appreciate everything.

Dr. Michael Persia. Thank you for being on my committee. Thank you for your advice and ideas to make my work better. Thank you for your amazing poultry classes. I guess I am a lucky person, because I have the best committee ever!

Dr. Frank Pierson. Thank you for your amazing and priceless poultry diseases class! Thank you very very much for your support during my hardest semester. Without it I might not pass this semester.

Dr. Samer El-Kadi. Thank you very much for your help with STAT. I do appreciate it.

The Egyptian ministry of higher education, Egyptian embassy staff, USAID and Cairo Initiative program directors. Thank you for all your help and support. Thank you very much for your scholarship program and for giving me this great opportunity to get my degree from such a great university, Virginia Tech! I do appreciate it.

Dr. Nada Tamim. Thank you for listening to me and for your great help.

Dr. Farid Stino. I cannot find enough words to thank you! Thank you very much for your encouragement and support both in Egypt and in the U.S. Thank you for believing in me. Thank

you for taking care of me everywhere and all the time as a beloved father. I am dedicating my thesis to you on your birthday, I hope you like your birthday gift!

Dr. Hossam Rushdi. Thank you for your great help with my paper work before traveling. Without you I would not be where I am today!

Dr. Rida Ali. Thank you for your great support.

Sunny. Thank you for teaching me all the lab work. Thank you for always being patient and answering my questions. Thank you for your time that you gave to me when you really needed it to finish your own work! You are one of the best lab seniors!

Shengchen. Thank you very much for teaching me all that I need. Thank you for your help all the time even during the weekends! You are such a great and helpful lab senior!

Wong lab members. Thank you for offering help, for your suggestions and comments during our lab meetings. You guys helped me a lot. Thank you **Shuai** for helping with STAT. Thank you **Javier** for your explanation regarding our shared interest (AvBDs). Thank you everyone.

Katie and Sydney. Thank you very much for your help and your time.

Friends. Thank you to all my friends in Egypt and in the U.S. Thank you for your help, support and prayers! Love you all!

Faculty and Staff at Virginia Tech. Thank you for all your help and assistance.

Mom. Thank you for giving me the genes of fighters! Yes! I got it from you my great mom and that is why I am still alive in spite of all those obstacles! Love you and miss you so much.

Dad. The best DAD ever! Finally your dream came true! I did it! Thank you for believing in me when everyone else did not! Thank you for supporting me until the last moment of your life! I wish you were here with me on this special day! I really miss you very much.

Ahmed El-Omda. I am delaying your acknowledgement because I do not have enough words to thank you! You are such a great husband and amazing friend! Thank you for delaying your graduation to be in the U.S. beside me and delaying your trip to attend my defense. Thank you for offering me such a peaceful life and a great love. Thank you for being always there for me. Thank you for sharing all the moments with me. Thank you for being patient and helpful. Thank you for taking care of me. Thank you for believing in me. Thanks for following my dream with me. I am lucky to have you! Guess what? We are finally going back home and will start our honeymoon after 8-months of marriage! It is funny but still exciting!! Love you!

TABLE OF CONTENTS

Abstract..ii

Dedication iii

Acknowledgements iv

Table of Contents..vii

List of Tables ix

List of Figures..x

List of Abbreviations xi

Chapter I: Introduction..1

Chapter II: Literature Review..3

Introduction..3

Avian immune system..3

Host defense peptides..6

Avian beta defensins 8

Antimicrobial role of AvBDs..10

Immunomodulatory role of AvBDs 10

Cathelicidins 11

Liver-expressed antimicrobial peptide 2 12

Applications 14

References..16

Chapter III: Comparative expression of host defense peptides in turkey poults 25

Introduction..25

Materials and Methods 27

Results..28

Discussion......29

References......34

Chapter IV: Epilogue 48

LIST OF TABLES

Table 1. Comparison between HDP classes……………………………………………………13

Table 2. qRT-PCR primers for turkey host defense peptides…………………………………..38

LIST OF FIGURES

Figure 1. AvBD1 expression at different developmental ages 39

Figure 2. AvBD2 expression at different developmental ages 40

Figure 3. AvBD8 expression at different developmental ages 41

Figure 4. AvBD9 expression at different developmental ages 42

Figure 5. AvBD10 expression at different developmental ages 43

Figure 6. AvBD13 expression at different developmental ages 44

Figure 7. Cath2 expression at different developmental ages 45

Figure 8. Cath3 expression at different developmental ages 46

Figure 9. LEAP-2 expression at different developmental ages 47

LIST OF ABBREVIATIONS

AMPs: antimicrobial peptides

APC: antigen-presenting cells

AvBDs: avian beta defensins

Bur: bursa of Fabricius

Caths: cathelicidins

DCs: dendritic cells

Duo: duodenum

GAL: gallinacins

GALT: gut-associated lymphoid tissue

GAPDH: Glyceraldehyde 3-phosphate dehydrogenase

HDPs: host defense peptides

Ile: ileum

Jej: jejunum

LEAP-2: liver-expressed antimicrobial peptide 2

LPS: lipopolysaccharide

MALT: mucosa-associated lymphoid tissue

NK cells: natural killer cells

qRT-PCR: quantitative real time polymerase chain reaction

Spl: spleen

Thy: thymus

CHAPTER I

INTRODUCTION

Turkey production has increased due to rising demand for turkey meat worldwide. According to the USDA, turkey meat consumption in the United States increased from 0.8 to 16.4 pounds per capita per year between 1910 and 2012. In 2012, the value of turkey meat production was over $5 billion, while in 2015 it was $5.71 billion (USDA National Agricultural Statistics Service (NASS), 2015). The American Livestock Breeds Conservancy (ALBC), Virginia Tech, and eight breeders and producers conducted a collaborative project to study the immune responses of commercial turkeys. The results showed that mortalities for commercial turkeys ranged from 13 to 93%, averaging 46% when they were challenged with hemorrhagic enteritis virus and *E. coli* (Larson, 2007). It was believed that the selection for performance parameters affected immune responses dramatically, hence studying the immune responses of turkeys is very important especially at an early age. Among poultry species, turkey poults are the second most sensitive to diseases, as they showed the greatest sensitivity after ducklings to dietary aflatoxins (Muller et al., 1970). This trend would be helped by more research to better understand and maintain turkey health especially in the younger stages of life. It is important to study the immune system of turkey poults as a fundamental basis for further research, which may help in finding new strategies to enhance the immune response in order to defend against challenges and keep poults as healthy as possible. The obvious criteria of healthy birds are their performance records, therefore, the routine performance data are good indicators of the health status of the birds, which would determine further action and care.

In this thesis, the literature review will briefly describe the function of the avian immune system, including both innate and adaptive immunity, with a focus on organs and cells involved in the immune response. Host defense peptides (HDPs) will then be discussed including their role in innate immunity, their mechanisms and classes. These peptides exist in avian species under the name “avian host defense peptides” and details about their expression, roles and applications will be presented. The experiment presented in this thesis includes investigation of HDP mRNA expression in healthy non-challenged turkey poults, a research area that has been better explored in chickens than in turkeys. Studying the patterns of HDP mRNA in young turkey poults is important as other components of their immune system are immature and the poults are more susceptible to disease.

CHAPTER II

LITERATURE REVIEW

Introduction

The immune system is responsible for defending against invading pathogens and other encountered antigens. In vertebrates, the immune system consists of two arms: the innate immune response and the adaptive immune response (Parkin and Cohen, 2001). Innate and adaptive immunity differ mainly in speed and specificity; however, there is constant interaction between the two. Like all body systems, the immune system is not fully functional during early development so characterization of the immune response is important in early stages of life.

Avian immune system

The immune system in birds is very similar to its counterpart in mammals. In all vertebrates including avian species, the immune system has two arms working cooperatively; innate response and adaptive response. Innate immunity, also called natural or native immunity, is the first line of defense thus it is an immediate but non-specific response. Innate immunity is provided by surface barriers such as skin and mucosal layers in addition to cellular and molecular defenses such as phagocytes including macrophages and heterophils, natural killer (NK) cells and host defense peptides (HDPs) (Abbas et al., 2012). Natural killer cells originate from lymphoid stem cells and can kill intracellular pathogens, especially virus-infected cells and neoplastic cells. Macrophages originate from bone marrow stem cells and play a key role in phagocytosis, cytokine production, antigen presentation and killing bacteria and tumor cells (Qureshi, 2003). Therefore, macrophages have important roles in both innate and adaptive immune responses. Heterophils are unique to avian species and represent the functional

counterparts of mammalian neutrophils, with the main differences being a lack of the myeloperoxidase enzyme, a lack of significant bactericidal activity by oxidative burst, and differing granule components from those in mammalian neutrophils (Montali, 1988). Heterophils are able to phagocytize and eliminate pathogens (Beal et al., 2006; Davison et al., 2011) and produce a number of defense peptides (Evans et al., 1994; Harwig et al., 1994; Potter, 2014).

Adaptive immunity, also called acquired immunity, takes longer to develop but is a more specialized response than innate immunity. The adaptive response needs time to allow lymphocytes (B cells and T cells) to clonally differentiate into highly specialized cells. T cells originate from the bone marrow, proliferate in the cortex of the thymus where they differentiate and mature, then are released from the thymus to peripheral tissues (Davison et al., 2011; Röhe, 2014). B cells proliferate and differentiate in the bursa of Fabricius, an organ unique to birds where B cells were first described, hence their name. B cells differentiate either into plasma cells that produce antibodies specific to the encountered antigens, or into memory cells, which "remember" those antigens and provide a swift and more robust response to subsequent encounters with the same pathogen (Abbas et al., 2012).

The avian immune system consists of a group of primary and secondary organs. The bursa of Fabricius and the thymus are primary organs while the spleen and gut-associated lymphoid tissues (GALT) are secondary organs. The bursa is an organ located at the dorsal end of the gut right above the cloaca and is the site for B cell differentiation. Mammals do not have a bursa but their B cells differentiate in the bone marrow. The thymus is a multi-lobed organ located on both sides of the neck and is the differentiation site for T cells (Sharma, 1991). The spleen is a secondary immune organ, whose function is filtration of blood debris like products of

phagocytosis and damaged cells. More importantly, the spleen is a critical organ for sampling of circulating antigens and inducing appropriate immune responses by facilitating the production, maturation and storage of lymphocytes (Smith and Hunt, 2004). The GALT is a part of the mucosa-associated lymphoid tissue (MALT) and an important secondary immune tissue, which can defend against enteric pathogens (Oláh and Glick, 1984; Muir et al., 2000). The GALT houses the largest populations of immune cells, more than any of the other lymphoid tissues (Röhe, 2014) and more than half of the total MALT lymphocyte populations (Yun et al., 2000; Bar-Shira et al., 2003).

The cells responsible for innate immunity are phagocytic cells such as heterophils, macrophages and dendritic cells (DCs). One or more of these cell populations could work on eliminating pathogens either directly or by processing and presenting antigens of phagocytized pathogens to other immune cells, thus they are known as antigen-presenting cells (APCs) (Kaspers and Kaiser, 2014). Heterophils are important granulated leukocytes with a broad spectrum antimicrobial activity against invading pathogens. The early identified heterophil-granule produced peptides were known as chicken heterophil antimicrobial peptides (CHP1, CHP2) and turkey heterophil antimicrobial peptides (THP1, THP2, THP3) (Evans et al., 1994; Harwig et al., 1994). Natural killer cells are large lymphocytes with electron dense granules (Potter, 2014). They are cytotoxic lymphocytes that do not require activation, and kill microbes through releasing toxic proteins to eliminate virus-infected cells and tumor cells (Sharma, 2003). The dendritic cells are also professional APCs that activate T cells and are key communicators between innate and adaptive immune responses (Steinman, 1991). AvBDs are produced by a number of cells including heterophils, macrophages, as well as enterocytes and other mucosal epithelial cells of the respiratory and urogenital tracts. While both Cath1 and Cath2 are

expressed mainly in bone marrow, Cath2 is highly expressed in heterophils (Potter, 2014).

Host defense peptides

Host defense peptides consist of a large group of small cationic (positively charged) and amphipathic peptides (hydrophilic and hydrophobic properties) (Zasloff, 2002; Wang, 2013), and are known to be conserved among different species of mammals, birds, insects and plants (Tossi et al., 2000; Lehrer and Ganz 2002; Zasloff, 2002; Zanetti, 2005). Host defense peptides are a component of the innate immune system and were originally called antimicrobial peptides (AMPs) due to their antimicrobial function. Recently, AMPs were renamed HDPs after discovering their other immunomodulatory properties (Cuperus et al., 2013). The mechanism by which HDPs kill microbes consists of two basic steps, binding to the cell then generating pores that lead to cell leakage and lysis (Yang et al., 2000). In general, HDPs have two major families: defensins and cathelicidins. Three subfamilies of defensins have been described, α, β and θ with only the β-defensins found in birds. Alpha-defensins are found only in mammals (Lynn and Bradley, 2007), while β-defensins are found in all vertebrate species (Lehrer and Ganz, 2002; Lynn et al., 2007). The third type called θ-defensins was found only in non-human primates (Tang et al., 1999). The α- and β-defensins are the main defensin subfamilies and they differ in the number of amino acids between the six conserved cysteines and the pairing of the cysteine residues via disulfide bonds (Ganz, 2003). There are three HDP classes in avian species: avian beta-defensins (AvBDs), cathelicidins (Caths), and liver-expressed antimicrobial peptide 2 (LEAP-2) (Cuperus et al., 2013).

The roles of HDPs are either immune modulation through recruitment of immune cells or direct killing of microbes (Hancock and Sahl, 2006). Direct killing occurs through the electrostatic interaction between the positive charge on the HDP and the negative charge on the

outer leaflet of the microbial cell. Generally, HDPs have a positive charge and consist of less than 100 amino acid residues with amphipathic properties. Thus, HDPs can interact with the microbial cell through hydrophobic interaction that allows the HDPs to diffuse between the lipid bilayers of the cell membrane, although it is a weak interaction. Further, HDPs can interact with animal cells only through the hydrophobic interaction, as these cells do not have a negative charge on the outer leaflet of the membrane and electrostatic interaction does not occur. Another reason why HDPs cannot effectively interact with the animal cell is that the animal cell membrane has cholesterol molecules that bind to the HDPs and prevent them from binding to the animal cell, as cholesterol is stable and interacts with peptides. That explains why HDPs are selective and can damage microbial cells but not animal cells (Zasloff, 2002). A number of mechanisms have been proposed for killing of microbes by HDPs such as depolarization of the normally energized bacterial membrane, binding to the microbial cell to make pores that cause cellular contents to leak, and activation of deadly processes like enhancing hydrolases to degrade the cell wall. In addition to disturbing membrane functions through scrambling of the normal distribution of lipids between the leaflets of bilayers, internalization of the peptide can critically damage intracellular targets (Kragol et al., 2001).

Most HDPs are produced as a prepropeptide consisting of an N-terminal signal sequence, a prosegment (a section of amino acids at one end of a protein molecule that assists in folding), and a C-terminal cationic peptide that exhibits antimicrobial activity after it is cleaved from the rest of the protein. These peptides have been grouped based on their primary structure, amino acid composition, and size (Reddy et al., 2004). In humans, β-defensins are expressed as a pro-peptide (an inactive peptide that can be turned into an active form), which goes through a

proteolytic cleavage process to release the mature peptide. However, the proteases related to the processing of β-defensins are not fully defined (Hemshekhar et al., 2016).

Avian beta defensins

Beta defensins represent a class of avian HDPs. They have a β-sheet structure and are cysteine-rich cationic peptides with three conserved disulfide bridges at Cys^1–Cys^5, Cys^2–Cys^4 and Cys^3–Cys^6 (Lehrer and Ganz, 2002; Klotman and Chang, 2006). In birds, only beta defensins are found, which were isolated from chicken heterophils (Evans et al., 1994) and originally called gallinacins (Gal), but later renamed avian beta defensins (AvBDs) (Lynn et al., 2007; Zhang and Sunkara, 2014). There are over 25 AvBDs, 14 were found in chickens and others were detected recently in the zebra finch (Hellgren and Ekblom, 2010). The 14 chicken AvBDs and 18 turkey AvBDs are clustered at the end of chromosome 3 (Dalloul et al., 2010; Hellgren and Ekblom, 2010).

There are differences in the structure between AvBDs; for example AvBD2 has no C-terminal amidation, different distribution of the cationic residues and is not amphipathic. AvBD1 and AvBD7 have C-terminal amidation, which helps these beta defensins to be more stable and more cationic and to have stronger antimicrobial activity (Bulet et al., 2004; Derache et al., 2009). Another example is the six cysteine bridges in AvBD11 instead of three, which might be caused by gene duplication (Herve-Grepinet et al., 2010).

Production of AvBDs is tissue-specific. Lynn et al. (2004) studied the expression of two groups; the first group included three known gallinacins (Gal-1 (AvBD1), Gal-2 (AvBD2) and Gal-3 (AvBD3), the second group included seven gallinacins (Gal-4 (AvBD6), Gal-5 (AvBD7), Gal-6 (AvBD9), Gal-7 (AvBD4), Gal-8 (AvBD10), Gal-9 (AvBD5), Gal-10 (AvBD12),

cathelicidin and LEAP-2 in 21 different tissues of healthy 3-week-old chickens. They reported that Gal-1 (AvBD1) and Gal-2 (AvBD2) were highly expressed in the lungs, bone marrow and testes, while moderately expressed in the bursa and intestine, but showed low expression in the cloaca, gall bladder, brain and pancreas. In addition, Gal-2 (AvBD2) showed low expression in the trachea, air sacs and spleen. Gal-3 (AvBD3) showed expression only in the tongue and bone marrow. Gal-4 (AvBD6), Gal-5 (AvBD7), Gal-7 (AvBD4) and Gal-8 (AvBD10) showed the same patterns of expression with high levels in the bone marrow and testes. Gal-6 (AvBD9) and Gal-8 (AvBD10) were highly expressed in the liver, gall bladder and kidneys. Gal-9 (AvBD5) was the only gene in the second group that was expressed in the tongue in addition to low expression in the esophagus, trachea, brain and bone marrow, while Gal-10 (AvBD12) showed low expression in the large intestine, kidneys and testes.

In another study, the expression of Gal-6 (AvBD9) was investigated in 23 tissues of healthy 6-week-old chickens (van Dijk et al., 2007). Gal-6 (AvBD9) was highly expressed in the trachea, esophagus, crop, bursa, kidney, liver, and muscle, while moderately expressed in the uropygial gland, brain, proventriculus, ovary, testis, and skin. Low expression was observed in the lung, thymus, spleen, and throughout the intestinal tract, and no expression was detected in the bone marrow. Ma et al. (2008) investigated Gal-8 (AvBD10) and Gal-9 (AvBD5) expression in 3-week-old chicks in 17 different tissues, and reported that Gal-9 (AvBD5) was highly expressed in the tongue and small intestine, but showed moderate expression in the proventriculus, lung, liver, heart, spleen, and thymus. Gal-8 (AvBD10) expression was high in the small intestine and liver while moderate in the tongue, and lung. In addition, Higgs et al. (2005) investigated the expression of two genes Gal-11 (AvBD13) and Gal-12 (AvBD8) in male chickens. Gal-11 (AvBD13) was highly expressed in the small intestine, liver, gall bladder and

spleen, while Gal-12 (AvBD8) was expressed only in the liver and gall bladder.

Antimicrobial roles of AvBDs: Antimicrobial activity against bacteria and fungi is first mediated through electrostatic interaction. Then the hydrophobic residues insert into the membrane and damage the microbial membrane, leading to death (Powers and Hancock, 2003; Ganz, 2004; Brogden, 2005). Therefore, the property of amphipathicity is very important. AvBD2, which is not amphipathic, can also kill bacteria (Derache et al., 2012). Such AvBDs kill Gram-positive bacteria more effectively, suggesting that the β-sheet structure may have an important role in killing those bacteria (Cuperus et al., 2013). On the contrary, since AvBD2 does not kill Gram-negative bacteria, this indicates that the electrostatic interaction through charge is more important to kill these bacteria (Cuperus et al., 2013).

Immunomodulatory role of AvBDs: AvBDs can bind directly to chemokine receptors to enhance expression of pro-inflammatory cytokines (Yang et al., 1999; Ganz, 2003); however, they have anti-inflammatory roles through blocking LPS-induced inflammation (Semple et al., 2011). AvBDs also can enhance wound healing and chemotaxis of monocytes. This function was attributed to AvBD2 in ducks (Soman et al., 2009a,b). Yurong and colleagues (2007) reported that AvBD13 can enhance chicken IgG and IgM serum levels, in addition to promoting spleen lymphocyte proliferation.

Beta defensins are conserved peptides between species but not all AvBDs possess the same function. The structure of AvBDs determines its function. AvBDs with positive charge are more important for killing Gram-negative bacteria, while the β-sheet structure is more important for killing Gram-positive bacteria.

Cathelicidins

Cathelicidins (Caths) contain cathelin-like domain short peptides, which are usually less than 40 amino acid peptides (Zanetti, 2005). Chicken Caths are clustered at the end of chromosome 2 (7.5 kB) (Xiao et al., 2006a). In chickens, there are four Caths, which are very similar in their amino acid content, especially Cath1 and Cath3 (van Dijk et al., 2005; Xiao et al., 2006a), while in turkeys only 3 Caths were found in NCBI databases (Cath2, Cath3, Cath-B1) (Aug, 2016). Like AvBDs, Caths have amphipathic properties, which enable them to interact with the bacterial membrane (Shai, 2002; Zasloff, 2002). Cath2 did not show any expression in the intestinal tissues of either healthy or infected tissues in chickens (van Dijk et al., 2009b, 2012) indicating that it may be expressed only by heterophils (Cuperus et al., 2013).

As a class of HDPs, Caths have antimicrobial activity against a broad spectrum of microbes, including Gram-positive and Gram-negative bacteria. This may be due to the presence of the N-terminal α-helical structure (Xiao et al., 2008; van Dijk et al., 2009a). However, it is not true that the high percentage of α-helical structure throughout the peptide will lead to more effective antimicrobial activity (Xiao et al., 2006b; Nan et al., 2012). Most Caths also have immunomodulatory activities, the main roles are blocking and neutralization of LPS and the direct immune stimulation by chemotaxis of immune cells (Bommineni et al., 2007). Caths can be divided into three subclasses based on the C-terminal mature HDPs; α-helical peptides (linear amphipathic peptide containing 23-37 amino acids), β-hairpin peptides (short chain of 12-18 amino acids formed by one or two disulfide bridges), and peptides which contain one or two amino acids such as tryptophan or proline (van Dijk et al., 2011). The α-helical structure is the most common structure and is the structure that was found in the known chicken Caths (Zanetti, 2005). Like AvBDs, there is also tissue-specific expression for Caths. Lynn and colleagues

(2004) studied the expression of chicken Cath, a novel gene at the time, in 21 different tissues of healthy 3-week-old birds. They observed high expression in the bursa, testes and bone marrow. In addition, chicken Cath was the only novel gene that showed expression in the gizzard.

Liver-expressed antimicrobial peptide 2

Liver-expressed antimicrobial peptide 2 (LEAP-2) is a 40 amino acid cationic peptide with two disulfide bridges at Cys17-Cys28 and Cys23-Cys33 (Hocquellet et al., 2010). The turkey LEAP-2 gene is located on chromosome 15 (Dalloul et al., 2010) while the chicken LEAP-2 gene is located on chromosome 13 (Townes et al., 2004), and both are similar structurally to mammalian LEAP-2 (Lynn et al., 2004). First isolated in 2003, LEAP-2 showed high expression in the human liver (Krause et al., 2003). Interestingly, LEAP-2 antimicrobial activity is more effective when the disulfide bridges are reduced compared to the original folded structure (Hocquellet et al., 2010). LEAP-2 showed tissue-specific expression in various chicken tissues including liver, intestine, gall bladder, kidney and reproductive organs; however, it was not expressed in either bone marrow or lymphoid organs (Cuperus et al., 2013). Like AvBDs and Caths, there is also tissue-specific expression for LEAP-2 in chickens. Lynn and colleagues (2004) reported high LEAP-2 expression in the liver, intestine, gall bladder and kidneys. Their results were consistent with human LEAP-2 expression in the liver, kidneys and colon (Krause et al., 2003).

Table 1. Comparison between HDP classes:

	Defensins	Cathelicidins	LEAP-2
Structure	Contain 18-45 amino acids β-sheet structure Cysteine-rich cationic peptides with three conserved disulfide bridges at Cys1–Cys5, Cys2–Cys4 and Cys3–Cys6 Six conserved cysteines that are oxidized to form three disulfide bonds. The structure determines function: Positive charge >> killing Gram- bacteria β-sheet structure >> killing Gram+ bacteria	Contain less than 40 amino acids Highly conserved 14 kDa N-terminal cathelin-like pro-domain, followed by a signal peptide and a C-terminal 'mature' peptide region.	Contains 40 amino acids Two disulfide bridges at Cys17-Cys28 and Cys23-Cys33
Function	β-defensins spectrum: Chickens: Gram+, Gram-, Fungi Turkey: Gram+, Gram- 14 Chicken AvBDs 18 Turkey AvBDs	Antimicrobial activity against: Bacteria, fungi, protozoa and enveloped viruses Multiple immune modulatory activities, such as chemotaxis towards blood cells and the binding and inactivation of endotoxin Binding to LPS Spectrum: Gram+, Gram- 4 Chicken Cathelicidins 3 Turkey Cathelicidins	Antimicrobial activity against: Bacteria, fungi and enveloped viruses

Applications

Commercial animal production including the turkey industry is constantly challenged by exposure to pathogens. Due to the rising issue of antibiotic resistance, enhancement of HDPs was proposed as an alternative strategy. Some research papers investigated the effect of feed additives on inducing HDP expression and enhancing the immune system. Sunkara et al. (2012) investigated the effect of butyrate, which is a short chain fatty acid derived from bacterial fermentation of undigested dietary fiber, on expression of HDPs in healthy chickens. They reported that butyrate enhanced the expression of several chicken HDPs but not all of them. AvBD3, AvBD4, AvBD8, AvBD9, AvBD10, AvBD14 and Cath B1 were upregulated after treatment with butyrate in HD11 cells, a chicken macrophage cell line. In chicken primary monocytes, AvBD3, AvBD5, AvBD14 and Cath B1 were upregulated after treatment with butyrate. On the other hand, AvBD2 in primary monocytes and AvBD7 in HD11 cells were slightly downregulated. Other HDPs, namely AvBD1, AvBD6 and Caths 1-3, were not changed after the butyrate treatment. Further, butyrate induced antimicrobial activity against *Salmonella* Enteritidis in the intestinal tract of chickens.

Tian and colleagues (2016) investigated the effect of dietary yeast β-glucans on the expression of HDPs in chickens with necrotic enteritis. They reported that in the β-glucans supplemented group, Cath2, AvBD4, and AvBD10 were upregulated at the early stage of infection, while Cath1, Cath2, and AvBD1 were upregulated at the late stage of infection. AvBD10 and LEAP-2 were downregulated at the late stage of infection. In conclusion, the yeast β-glucans enhanced the expression of some HDPs and improved the health of challenged chickens by inhibiting *C. perfringens* growth, improving villi height and villi height to crypt depth ratio, and inducing humoral immunity and expression of host defense peptides. This led to

increased body weight gain and improved feed conversion ratio in the supplemented infected group compared to the non-supplemented infected group.

This brief literature review presents the significance of the HDPs and their potential role in immune responses of young birds, and highlights the importance of turkey production especially in the United States. HDPs have not been as well studied in turkeys as in chickens, and this project is aimed at documenting expression profiles of HDPs that may help fill a gap in that knowledge area. Understanding the expression patterns of HDPs in healthy turkey poults may provide baseline information so that further research can be done to investigate new strategies to enhance the poult's immune system, particularly innate immunity.

References

Abbas, A. K., A. H. Lichtman, and S. Pillai. 2012. Basic immunology: functions and disorders of the immune system. Elsevier Health Sciences.

Bar-Shira, E., D. Sklan, and A. Friedman. 2003. Establishment of immune competence in the avian GALT during the immediate post-hatch period. Dev. Comp. Immunol. 27:147-157.

Beal, R. K., C. Powers, T. F. Davison, and A. L. Smith. 2006. Immunological development of the avian gut. Pages 85-102 in: Avian Gut Function in Health and Disease. Iowa State Press, Ames, IA.

Bommineni, Y. R., H. Dai, Y. X. Gong, J. L. Soulages, S. C. Fernando, U. DeSilva, O. Prakash, and G. Zhang. 2007. Fowlicidin 3 is an α-helical cationic host defense peptide with potent antibacterial and lipopolysaccharide-neutralizing activities. FEBS J. 274:418-428.

Brogden, K. A. 2005. Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? Nat. Rev. Microbiol. 3:238-250.

Bulet, P., R. Stöcklin, and L. Menin. 2004. Antimicrobial peptides: from invertebrates to vertebrates. Immunol. Rev. 198:169-184.

Cuperus, T., M. Coorens, A. van Dijk, and H. P. Haagsman. 2013. Avian host defense peptides. Dev. Comp. Immunol. 41:352-369.

Dalloul, R. A., J. A. Long, A. V. Zimin, L. Aslam, K. Beal, L. A. Blomberg, P. Bouffard, D. W. Burt, O. Crasta, R. P. Crooijmans, et al. 2010. Multi-platform next-generation sequencing of the domestic turkey (*Meleagris gallopavo*): genome assembly and analysis. PLoS Biol. 8:e1000475.

Davison, F., B. Kaspers, K. A. Schat, and P. Kaiser. 2011. Avian immunology. Academic Press.

Derache, C., V. Labas, V. Aucagne, H. Meudal, C. Landon, A. F. Delmas, T. Magallon, and A. C. Lalmanach. 2009. Primary structure and antibacterial activity of chicken bone marrow-derived β-defensins. Antimicrob. Agents Chemother. 53:4647-4655.

Derache, C., H. Meudal, V. Aucagne, K. J. Mark, M. Cadène, A. F. Delmas, A. C. Lalmanach, and C. Landon. 2012. Initial insights into structure-activity relationships of avian defensins. J. Biol. Chem. 287:7746-7755.

Evans, E. W., G. G. Beach, J. Wunderlich, and B. G. Harmon. 1994. Isolation of antimicrobial peptides from avian heterophils. J. Leukoc. Biol. 56:661-665.

Ganz, T. 2003. Defensins: antimicrobial peptides of innate immunity. Nat. Rev. Immunol. 3:710-720.

Ganz, T. 2004. Defensins: antimicrobial peptides of vertebrates. C. R. Biol. 327:539-549.

Hancock, R. E., and H.-G. Sahl. 2006. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. Nat. Biotechnol. 24:1551-1557.

Harwig, S. S., K. M. Swiderek, V. N. Kokryakov, L. Tan, T. D. Lee, E. A. Panyutich, G. M. Aleshina, O. V. Shamova, and R. I. Lehrer. 1994. Gallinacins: cysteine rich antimicrobial peptides of chicken leukocytes. FEBS Lett. 342:281-285.

Hellgren, O., and R. Ekblom. 2010. Evolution of a cluster of innate immune genes (β-defensins) along the ancestral lines of chicken and zebra finch. Immunome Res. 6:1.

Hemshekhar, M., V. Anaparti, and N. Mookherjee. 2016. Functions of cationic host defense peptides in immunity. Pharmaceuticals 9:40.

Hervé-Grépinet, V., S. Réhault-Godbert, V. Labas, T. Magallon, C. Derache, M. Lavergne, J. Gautron, A. C. Lalmanach, and Y. Nys. 2010. Purification and characterization of avian

β-defensin 11, an antimicrobial peptide of the hen egg. Antimicrob. Agents Chemother. 54:4401-4409.

Higgs, R., D. J. Lynn, S. Gaines, J. McMahon, J. Tierney, T. James, A. T. Lloyd, G. Mulcahy, and C. O'Farrelly. 2005. The synthetic form of a novel chicken β-defensin identified in silico is predominantly active against intestinal pathogens. Immunogenetics 57:90-98.

Hocquellet, A., B. Odaert, C. Cabanne, A. Noubhani, W. Dieryck, G. Joucla, C. Le Senechal, M. Milenkov, S. Chaignepain, and J. M. Schmitter. 2010. Structure–activity relationship of human liver-expressed antimicrobial peptide 2. Peptides 31:58-66.

Kaspers, B., and P. Kaiser. 2014. Avian antigen-presenting cells. Pages 169-188 in: Avian Immunology, 2nd Ed. Academic Press, San Diego, CA.

Klotman, M. E., and T. L. Chang. 2006. Defensins in innate antiviral immunity. Nat. Rev. Immunol. 6:447-456.

Kragol, G., and L. Otvos. 2001. Orthogonal solid-phase synthesis of tetramannosylated peptide constructs carrying three independent branched epitopes. Tetrahedron 57:957-966.

Krause, A., R. Sillard, B. Kleemeier, E. Klüver, E. Maronde, J. R. Conejo‐García, W. G. Forssmann, P. Schulz‐Knappe, M. C. Nehls, and F. Wattler. 2003. Isolation and biochemical characterization of LEAP-2, a novel blood peptide expressed in the liver. Protein Sci. 12:143-152.

Lehrer, R. I., and T. Ganz. 2002. Defensins of vertebrate animals. Curr. Opin. Immunol. 14:96-102.

Lynn, D. J., and D. G. Bradley. 2007a. Discovery of α-defensins in basal mammals. Dev. Comp. Immunol. 31:963-967.

Lynn, D. J., R. Higgs, S. Gaines, J. Tierney, T. James, A. T. Lloyd, M. A. Fares, G. Mulcahy, and C. O'Farrelly. 2004. Bioinformatic discovery and initial characterisation of nine novel antimicrobial peptide genes in the chicken. Immunogenetics 56:170-177.

Lynn, D. J., R. Higgs, A. T. Lloyd, C. O'Farrelly, V. Hervé-Grépinet, Y. Nys, F. S. Brinkman, P. L. Yu, A. Soulier, and P. Kaiser. 2007b. Avian beta-defensin nomenclature: a community proposed update. Immunol. Lett. 110:86-89.

Ma, D. Y., S. W. Liu, Z. X. Han, Y. J. Li, and A. S. Shan. 2008. Expression and characterization of recombinant gallinacin-9 and gallinacin-8 in *Escherichia coli*. Protein Expres. Purif. 58:284-291.

Montali, R. J. 1988. Comparative pathology of inflammation in the higher vertebrates (reptiles, birds and mammals). J. Comp. Pathol. 99:1-26.

Muir, W. I., W. L. Bryden, and A. J. Husband. 2000. Immunity, vaccination and the avian intestinal tract. Dev. Comp. Immunol. 24:325-342.

Muller, R., C. Carlson, G. Semeniuk, and G. Harshfield. 1970. The response of chicks, ducklings, goslings, pheasants and poults to graded levels of aflatoxins. Poult. Sci. 49:1346-1350.

Nan, Y. H., J. K. Bang, B. Jacob, I.-S. Park, and S. Y. Shin. 2012. Prokaryotic selectivity and LPS-neutralizing activity of short antimicrobial peptides designed from the human antimicrobial peptide LL-37. Peptides 35:239-247.

Oláh, I., and B. Glick. 1984. Meckel's diverticulum. I. Extramedullary myelopoiesis in the yolk sac of hatched chickens (*Gallus domesticus*). Anat. Rec. 208:243-252.

Parkin, J., and B. Cohen. 2001. An overview of the immune system. The Lancet 357:1777-1789.

Potter, T. D. 2014. Bacterial profiles and ex vivo effects of *Salmonella* Heidelberg on leukocyte function in turkey purebred lines. M.S. Thesis. Virginia Tech.

Powers, J. P. S., and R. E. W. Hancock. 2003. The relationship between peptide structure and antibacterial activity. Peptides 24:1681-1691.

Qureshi, M. A. 2003. Avian macrophage and immune response: an overview. Poult. Sci. 82:691-698.

Reddy, K., R. Yedery, and C. Aranha. 2004. Antimicrobial peptides: premises and promises. Int. J. Antimicrob. Agents 24:536-547.

Röhe, I. 2014. Effects of feed structure on animal performance, gastrointestinal morphology, gut-associated lymphoid tissue and jejunal glucose transport in laying hens. Ph.D. Dissertation. Freie Universität Berlin.

Semple, F., H. MacPherson, S. Webb, S. L. Cox, L. J. Mallin, C. Tyrrell, G. R. Grimes, C. A. Semple, M. A. Nix, and G. L. Millhauser. 2011. Human β-defensin 3 affects the activity of pro-inflammatory pathways associated with MyD88 and TRIF. Eur. J. Immunol. 41:3291-3300.

Shai, Y. 2002. Mode of action of membrane active antimicrobial peptides. Peptide Sci. 66:236-248.

Sharma, J. M. 1991. Overview of the avian immune system. Vet. Immunol. Immunopathol. 30:13-17.

Sharma, J. M. 2003. The avian immune system. Diseases of Poultry, 11th ed. YM Saif, ed. The Iowa State University Press, Ames, IA:5-16.

Smith, K. G., and J. L. Hunt. 2004. On the use of spleen mass as a measure of avian immune system strength. Oecologia 138:28-31.

Soman, S. S., D. S. Arathy, and E. Sreekumar. 2009a. Discovery of Anas platyrhynchos avian β-defensin 2 (Apl_AvBD2) with antibacterial and chemotactic functions. Mol. Immunol. 46:2029-2038.

Soman, S. S., S. Nair, A. Issac, D. S. Arathy, K. P. Niyas, M. Anoop, and E. Sreekumar. 2009b. Immunomodulation by duck defensin, Apl_AvBD2: In vitro dendritic cell immunoreceptor (DCIR) mRNA suppression, and B-and T-lymphocyte chemotaxis. Mol. Immunol. 46:3070-3075.

Steinman, R. M. 1991. The dendritic cell system and its role in immunogenicity. Annu. Rev. Immunol. 9:271-296.

Sunkara, L. T., W. Jiang, and G. Zhang. 2012. Modulation of antimicrobial host defense peptide gene expression by free fatty acids. PLoS One 7:e49558.

Tang, Y. Q., J. Yuan, G. Ösapay, K. Ösapay, D. Tran, C. J. Miller, A. J. Ouellette, and M. E. Selsted. 1999. A cyclic antimicrobial peptide produced in primate leukocytes by the ligation of two truncated α-defensins. Science 286:498-502.

Tian, X., Y. Shao, Z. Wang, and Y. Guo. 2016. Effects of dietary yeast β-glucans supplementation on growth performance, gut morphology, intestinal *Clostridium perfringens* population and immune response of broiler chickens challenged with necrotic enteritis. Anim. Feed Sci. Technol. 215:144-155.

Tossi, A., L. Sandri, and A. Giangaspero. 2000. Amphipathic, α-helical antimicrobial peptides. Peptide Sci. 55:4-30.

Townes, C. L., G. Michailidis, C. J. Nile, and J. Hall. 2004. Induction of cationic chicken liver-expressed antimicrobial peptide 2 in response to *Salmonella enterica* infection. Infect. Immun. 72:6987-6993.

USDA National Agricultural Statistics Service. 2015. National Statistics for Turkeys, Productions measures in $ US. Last Modified Apr.28, 2016. http://www.nass.usda.gov/Charts_and_Maps/index.php

van Dijk, A., M. Herrebout, M. H. G. Tersteeg-Zijderveld, J. L. M. Tjeerdsma-van Bokhoven, N. Bleumink-Pluym, A. J. M. Jansman, E. J. A. Veldhuizen, and H. P. Haagsman. 2012. *Campylobacter jejuni* is highly susceptible to killing by chicken host defense peptide cathelicidin-2 and suppresses intestinal cathelicidin-2 expression in young broilers. Vet. Microbiol. 160:347-354.

Van Dijk, A., E. Molhoek, F. Bikker, P.-L. Yu, E. Veldhuizen, and H. Haagsman. 2011. Avian cathelicidins: Paradigms for the development of anti-infectives. Vet. Microbiol. 153:27-36.

Van Dijk, A., E. M. Molhoek, E. J. A. Veldhuizen, J. L. M. Tjeerdsma-van Bokhoven, E. Wagendorp, F. Bikker, and H. P. Haagsman. 2009a. Identification of chicken cathelicidin-2 core elements involved in antibacterial and immunomodulatory activities. Mol. Immunol. 46:2465-2473.

Van Dijk, A., M. H. G. Tersteeg-Zijderveld, J. L. M. Tjeerdsma-van Bokhoven, A. J. M. Jansman, E. J. A. Veldhuizen, and H. P. Haagsman. 2009b. Chicken heterophils are recruited to the site of *Salmonella* infection and release antibacterial mature Cathelicidin-2 upon stimulation with LPS. Mol. Immunol. 46:1517-1526.

van Dijk, A., E. J. A. Veldhuizen, S. I. C. Kalkhove, J. L. M. Tjeerdsma-van Bokhoven, R. A. Romijn, and H. P. Haagsman. 2007. The β-defensin gallinacin-6 is expressed in the chicken digestive tract and has antimicrobial activity against food-borne pathogens. Antimicrob. Agents Chemother. 51:912-922.

Van Dijk, A., E. J. A. Veldhuizen, A. J. A. M. van Asten, and H. P. Haagsman. 2005. CMAP27, a novel chicken cathelicidin-like antimicrobial protein. Vet. Immunol. Immunopathol. 106:321-327.

Wang, G. 2013. Database-guided discovery of potent peptides to combat HIV-1 or superbugs. Pharmaceuticals 6:728-758.

Xiao, Y., Y. Cai, Y. R. Bommineni, S. C. Fernando, O. Prakash, S. E. Gilliland, and G. Zhang. 2006a. Identification and functional characterization of three chicken cathelicidins with potent antimicrobial activity. J. Biol. Chem. 281:2858-2867.

Xiao, Y., H. Dai, Y. R. Bommineni, J. L. Soulages, Y. X. Gong, O. Prakash, and G. Zhang. 2006b. Structure–activity relationships of fowlicidin-1, a cathelicidin antimicrobial peptide in chicken. FEBS J. 273:2581-2593.

Xiao, Y., A. I. Herrera, Y. R. Bommineni, J. L. Soulages, O. Prakash, and G. Zhang. 2008. The central kink region of fowlicidin-2, an α-helical host defense peptide, is critically involved in bacterial killing and endotoxin neutralization. Innate Immun. 1:268-280.

Yang, D., O. B. S. N. Chertov, S. N. Bykovskaia, Q. Chen, M. J. Buffo, J. Shogan, M. Anderson, J. M. Schröder, J. M. Wang, and O. M. Z. Howard. 1999. β-defensins: linking innate and adaptive immunity through dendritic and T cell CCR6. Science 286:525-528.

Yang, L., T. M. Weiss, R. I. Lehrer, and H. W. Huang. 2000. Crystallization of antimicrobial pores in membranes: magainin and protegrin. Biophys. J. 79:2002-2009.

Yun, C. H., H. S. Lillehoj, and E. P. Lillehoj. 2000. Intestinal immune responses to coccidiosis. Dev. Comp. Immunol. 24:303-324.

Yurong, Y., J. Yibao, S. Ruiping, P. Kaisong, Z. Xuemei, Y. Qingqiang, W. Decheng, L. Tianlong, and B. Huihui. 2007. Effects of Gal-13 on the content of immunoglobulin,

proliferation of lymphocyte and antibody titers after vaccination with infectious bursal disease virus vaccine in chickens. Asian Austral. J. Ani. Sci. 20:405.

Zanetti, M. 2005. The role of cathelicidins in the innate host defenses of mammals. Curr. Issues Mol. Biol. 7:179-196.

Zasloff, M. 2002. Antimicrobial peptides of multicellular organisms. Nature 415:389-395.

Zhang, G., and L. T. Sunkara. 2014. Avian antimicrobial host defense peptides: from biology to therapeutic applications. Pharmaceuticals 7:220-247.

CHAPTER III

Comparative Expression of Host Defense Peptides in Turkey Poults

Introduction

The immune system is responsible for defending against pathogens and other environmental insults. In vertebrates, the immune system consists of two main branches: innate and adaptive immunity, which broadly differ in response time and specificity (Parkin and Cohen, 2001). Innate immunity is the first line of defense, thus it is immediate but lacks high specificity. In contrast, the adaptive immune response requires more time to develop but is highly specific in its defense against pathogens, with both innate and adaptive immunity working in parallel. Host defense peptides (HDPs) are one of the innate immunity components and also help in the recruitment of immune cells to enhance the adaptive immune response. HDPs consist of a large group of small peptides, which are cationic and amphipathic peptides with direct antimicrobial activities and immunomodulatory properties (Zasloff, 2002; van Dijk et al., 2008; Wang, 2013). The antimicrobial activity of HDPs helps to defend against bacteria, fungi, protozoa and enveloped viruses, while the immunomodulatory properties help in boosting adaptive immunity through chemotaxis of lymphocytes. The mechanism by which HDPs kill microbes consists of two basic stages: first binding to the cell and then making pores that lead to cell leakage and lysis (Yang et al., 2000). Due to their antimicrobial function, HDPs were originally called antimicrobial peptides and then recently renamed HDPs after discovering their immunomodulatory properties (Cuperus et al., 2013).

HDPs have two major families: defensins and cathelicidins. Three subfamilies of defensins have been described, α, β and θ with only the β-defensins found in birds. The α- and

β-defensins are the main defensin subfamilies and they differ in the number of amino acids between the six conserved cysteines and the pairing of the cysteine residues via disulfide bonds (Ganz, 2003). In avian species, there are three HDP classes: avian beta-defensins (AvBDs), cathelicidins (Caths), and liver-expressed antimicrobial peptide 2 (LEAP-2) (Cuperus et al., 2013). The 14 AvBDs differ in their chemical structure, mainly amino acid sequence and composition (Klüver et al., 2006), but usually consist of 14-45 amino acids. AvBDs were first isolated from chicken heterophils (Evans et al., 1994), and were originally called gallinacins and then renamed as AvBDs (Lynn et al., 2007). The second class is Caths, which are short peptides consisting of less than 40 amino acids with a highly conserved cathelin-like domain (Zanetti, 2005). Four different Caths have been described in birds; however, their numbers differ between and within species (van Dijk et al., 2011). Since Caths are HDPs, they have the same mode of action and mechanisms as AvBDs. The third class is LEAP-2, which is a peptide consisting of 40 amino acids with two disulfide bridges and is highly expressed in the liver and intestine (Hocquellet et al., 2010). LEAP-2 has a unique primary structure yet is similar to other HDPs in terms of the positively charged amino acid residues and the multiple disulfide bonds (Krause et al., 2003).

Avian HDPs are not well characterized in turkeys. The objective of this study was to profile the gene expression patterns of nine HDPs (AvBD1, AvBD2, AvBD8, AvBD9, AvBD10, AvBD13, Cath2, Cath3, LEAP-2) in immune organs (thymus, spleen, bursa) and intestinal tissues (duodenum, jejunum, ileum) of commercial turkey poults at different early developmental stages (D0, D7, D14, D21, D28), which represent a critical period in the poult life when its immune system is not fully developed.

Materials and Methods

Tissue Sampling

Tissue samples (n=6) were previously collected at day of hatch (D0), D7, D14, D21, and D28 from thymus, spleen, bursa, duodenum, jejunum and ileum from commercial broad-breasted male turkey poults (AgForte, Harrisonburg, VA). The birds were raised on large floor pens using fresh wood shavings with commercial starter poult feed and water provided ad libitum from hatch to four weeks of age. The birds were uninfected and were not vaccinated, and assumed to be healthy (Weintraut et al., 2016). The samples were stored in RNA*later* (Life Technologies, Grand Island, NY) until RNA extraction.

RNA Extraction and Relative qPCR

My starting point was total RNA extraction from tissues using the RNeasy Plus Universal Mini Kit (Qiagen, Valencia, CA) with QIAzol Lysis Reagent (Qiagen) according to the manufacturer's instructions. The RNA concentration was measured using a NanoDrop 1000 (Thermo Scientific). cDNA was synthesized from 2 µg total RNA using the High Capacity Reverse Transcription cDNA kit (Applied Biosystems, Foster City, CA), then diluted 1:20 with RNase-free water. qRT-PCR reactions were run using 1 µL diluted cDNA, 5 µL Fast SYBR Green Master Mix (Applied Biosystems), 1 µL of both forward and reverse primers, and RNase-free water to reach a total volume of 10 µL using an ABI 7500 Fast Real-Time PCR instrument (Applied Biosystems, Inc). The manufacturer's default program was used (95°C for 20 s, 40 cycles of 95°C for 3 s and 60°C for 30 s). The primers for the nine target genes were designed by Primer Express (v3.0) software (Applied Biosystems) and are listed in Table 2.

Fold change was calculated using the relative quantification method ($2^{-\Delta\Delta C_T}$ method) (Livak and

Schmittgen, 2001). GAPDH was used as the reference gene and the average value for D0 thymus was used as the calibrator in all examined tissues because it was the lowest value. JMP Pro 12.0 software (SAS Inc., Cary, NC) was used for statistical analysis. Fold change data were analyzed by one-way ANOVA and Tukey's test with significance considered at $P < 0.05$.

Results

Overall, AvBDs and Caths showed greater expression in the immune tissues than the intestinal tissues, whereas LEAP-2 showed greater expression in the intestine than immune tissues. The AvBDs showed both tissue- and development-specific expression. AvBD1 expression in the thymus was low at D0, D7 and D14 and increased at D21 (Figure 1). While in the spleen, AvBD1 expression was high at D0 and declined at D7, D14 and D28. In the duodenum, AvBD1 expression was low at D0, D7, D14 and increased at D28, with an intermediate level at D21. No differences were detected between different time points for bursa, jejunum or ileum. The expression profile of AvBD2 was similar to that of AvBD1 in the thymus and spleen but was initially low in the thymus at D0 and D7 and increased at D21 (Figure 2). In the spleen, AvBD2 was high at D0 and then declined to D7, D14, D21, and D28. There were no differences for bursa or the three intestinal segments. For AvBD8, there was differential expression in only the duodenum (Figure 3) expression was low at D0, D7, and D14 and increased at D28 with D21 intermediate; this was similar to the expression pattern observed for AvBD1 in the duodenum. Expression of AvBD9 in the thymus was the same as for AvBD1, with low expression at D0, D7, and D14 and greater expression at D21 (Figure 4). There were no differences in the other tissues examined. AvBD10 expression in the thymus was low at D0, D7 and D14 and increased at D21 (Figure 5). In the small intestine, AvBD10 expression was

greatest at D0 and then declined at D7, D14, and D21 in the duodenum, jejunum and ileum, and D28 in the jejunum and ileum. Like AvBD1 and AvBD9, AvBD13 expression in the thymus was low at D0, D7, and D14 and increased to D21 (Figure 6). In the duodenum, AvBD13 expression was low at D0 and D7 and increased at D28.

The cathelicidins Cath2 and Cath3 also showed tissue- and development-specific expression. Both Cath2 and Cath3 showed low expression in the thymus at D0, which increased to D14 and then declined at D21 and D28 (Figures 7 and 8). In the spleen, expression of Cath2 and Cath3 was the greatest at D0, which rapidly declined to D7 through D28. In the bursa, there was low expression of Cath3, which increased from D7 to D14 and then declined to D21. In the small intestine, Cath2 expression was low at D0 and D7 and increased at D21 in the ileum.

LEAP-2 was expressed greater in the intestine than the immune tissues and showed a more complex pattern of tissue- and development-specific expression (Figure 9). In the thymus, LEAP-2 increased from D0 to D7 and then declined at D14 through D28. In the bursa, there was low expression of LEAP-2, which increased from D14 to D21 and D28. In the small intestine, LEAP-2 was low at D0, increased at D7, decreased at D14 and D21, and increased again at D28 in the duodenum. In the jejunum, LEAP-2 expression increased from D7 to D14 and reached the greatest level at D21 and D28; whereas in the ileum, LEAP-2 increased from D7 to D28.

Discussion

In this study, the expression of nine HDPs was assessed. The objective of this study was to investigate the developmental mRNA expression pattern of the HDPs in healthy turkey poults at weekly intervals in intestinal and immune tissues. The hypothesis was that expression of HDPs would decline as the birds age, based on the assumption that adaptive immunity

progressively develops with age (Achanta et al., 2012). The thymus, bursa and spleen are important immune organs that contribute to host immunity. The thymus and bursa are primary immune organs while the spleen is a secondary immune organ. The function of the thymus and bursa is to help with production and differentiation of T cells and B cells, respectively. In addition to its function in blood filtration, the spleen is a key immune organ for systemic antigen sampling and mounting appropriate humoral and/or cell-mediated immune responses (Smith and Hunt, 2004).

In this study, the thymus and spleen expressed high levels of HDPs in a tissue- and temporal-specific manner. Based on these expression profiles, spleen showed the greatest AvBDs expression between the examined tissues. Our results indicated that expression of AvBDs was greater in the immune organs than the intestinal tissues. AvBD1 and AvBD2 were highly expressed in the turkey spleen at D0, which declined at D7 and remained low until D28, which corresponds to the expected pattern. Hong and colleagues (2012) observed high expression of AvBD1, AvBD2, AvBD4, AvBD5, AvBD6 and AvBD7 in the spleen of uninfected Ross and Cobb chickens at 20 days of age. Because expression was examined at only a single time point, it is unknown if AvBD expression was greater at earlier time points in broilers. In the current study, except for AvBD8, the mRNA expression of other examined AvBDs in the thymus showed the highest expression at D21, which did not significantly differ from D28 contrary to what was expected. While in the spleen, AvBD1, and AvBD2 expression seemed predictable as it showed the greatest expression at D0 and lower expression at all other time points. This indicated that AvBD1 and AvBD2 may have a specific role in the early production and differentiation of B cells and T cells in the spleen other than regular activation and recruitment.

The small intestine not only plays the major role of nutrient digestion and absorption, but also is the largest lymphoid tissue and is key to gut immunity and health. The gut-associated lymphoid tissues (GALT) are part of the mucosa-associated lymphoid tissues (MALT) and contain the highest number of leukocytes among all lymphoid tissues (Röhe, 2014). The GALT comprise several types of lymphoid elements harboring both innate and adaptive immune cells necessary for dealing with pathogens. In addition, the intestinal epithelial cells play an important role in the innate immune response via the production of HDPs and communication signals like cytokines and chemokines. Ramasamy et al. (2012) reported that in the ileum of 4-day old non-infected chickens, AvBD13 and AvBD14 were significantly expressed; in contrast, the expression of turkey AvBD13 in the ileum was very low. Interestingly, only AvBD10 in turkeys followed the expected pattern in the intestinal tissues starting with the greatest expression at D0 then declining with age. This indicates that AvBD10 may act more during early infection in the small intestine while AvBD1 and AvBD8 may function in later infection.

LEAP-2 has a different structure and exhibited different patterns from AvBDs as manifested by greater expression in the intestinal tissues than the immune tissues. LEAP-2 is highly conserved among vertebrates (Howard et al., 2010). In catfish, LEAP-2 had high expression levels in the intestine and nine other tissues (Bao et al., 2006). In the thymus, LEAP-2 showed early upregulation from D0 to D7, followed by Cath2 and Cath3 that were upregulated at D14, and then AvBDs that were upregulated at D21. LEAP-2 expression showed a dramatic increase from D0 to D7 in the duodenum, with greater expression in the duodenum than the jejunum and ileum. The latter result is similar to that observed for LEAP-2 expression in the duodenum, jejunum and ileum of 28 day old non-infected broilers (Su et al., 2015). The high expression of LEAP-2 in the small intestine implies a possible function in regulating local

microbial populations, which could explain its low expression in the thymus, spleen and bursa. Cath2 and Cath3 expression in the intestinal tissues was similar to that found in previous studies. Cath2 did not show any expression in the gastrointestinal tissues of either healthy or infected birds, which indicates that the expression may be restricted to the heterophils only and not the epithelial tissues (van Dijk et al., 2009, 2012).

The same intestinal tissues of this experiment were used in a previous study by Weintraut and colleagues (2016) to profile the mRNA expression of nutrient transporters in males and female turkeys. The expression of most of the transporters was greater in females than males. While female tissues were not profiled in this study, we speculate differences between males and females to also exist in expression patterns of HDPs, as immune competence often declines upon selection for higher performance traits such as body weight, an aspect of potential future research.

Some of our results showed statistical differences which is not necessarily important from the biological stand point. Examples include the statistical significant differences in Cath2 expression between ages in the ileum and in Cath3 and LEAP-2 expression between ages in the bursa. These tissues showed statistically significant differences between different ages; however, the expression was lower than 1-fold change which may not be biologically relevant. Other results showed large differences without statistical significance due to the large variation between the birds in these specific data sets, therefore rendering some of the interpretation more difficult.

In conclusion, due to the commercial importance of turkeys and their sensitivity to diseases especially in the first few weeks of age, this study represents an important foundation for understanding expression patterns of HDPs and innate immunity in turkey poults. These

findings can be used in further research to improve the health of turkeys at early stages of life through the use of dietary components (e.g. butyrate, probiotics) to regulate HDP expression and accordingly enhance the immune system (Trebichavský and Šplíchal, 2006; Sunkara et al., 2012).

References

Achanta, M., L. T. Sunkara, G. Dai, Y. R. Bommineni, W. Jiang, and G. Zhang. 2012. Tissue expression and developmental regulation of chicken cathelicidin antimicrobial peptides. J. Anim. Sci. Biotech. 3:1.

Bao, B., E. Peatman, P. Xu, P. Li, H. Zeng, C. He, and Z. Liu. 2006. The catfish liver-expressed antimicrobial peptide 2 (LEAP-2) gene is expressed in a wide range of tissues and developmentally regulated. Mol. Immmunol. 43:367-377.

Cuperus, T., M. Coorens, A. van Dijk, and H. P. Haagsman. 2013. Avian host defense peptides. Dev. Comp. Immunol. 41:352-369.

Derache, C., H. Meudal, V. Aucagne, K. J. Mark, M. Cadène, A. F. Delmas, A. C. Lalmanach, and C. Landon. 2012. Initial insights into structure-activity relationships of avian defensins. J. Biol. Chem. 287:7746-7755.

Evans, E. W., G. G. Beach, J. Wunderlich, and B. G. Harmon. 1994. Isolation of antimicrobial peptides from avian heterophils. J. Leukoc. Biol. 56:661-665.

Ganz, T. 2003. Defensins: antimicrobial peptides of innate immunity. Nat. Rev. Immunol. 3:710-720.

Hocquellet, A., B. Odaert, C. Cabanne, A. Noubhani, W. Dieryck, G. Joucla, C. Le Senechal, M. Milenkov, S. Chaignepain, and J. M. Schmitter. 2010. Structure–activity relationship of human liver-expressed antimicrobial peptide 2. Peptides 31:58-66.

Hong, Y. H., W. Song, S. Lee, and H. S. Lillehoj. 2012. Differential gene expression profiles of β-defensins in the crop, intestine, and spleen using a necrotic enteritis model in 2 commercial broiler chicken lines. Poult. Sci. 91:1081-1088.

Howard, A., C. Townes, P. Milona, C. J. Nile, G. Michailidis, and J. Hall. 2010. Expression and functional analyses of liver-expressed antimicrobial peptide 2 (LEAP-2) variant forms in human tissues. Cell. Immunol. 261:128-133.

Klüver, E., K. Adermann, and A. Schulz. 2006. Synthesis and structure‐activity relationship of β-defensins, multi-functional peptides of the immune system. J. Pept. Sci. 12:243-257.

Krause, A., R. Sillard, B. Kleemeier, E. Klüver, E. Maronde, J. R. Conejo ‐ García, W. G. Forssmann, P. Schulz ‐ Knappe, M. C. Nehls, and F. Wattler. 2003. Isolation and biochemical characterization of LEAP-2, a novel blood peptide expressed in the liver. Protein Sci. 12:143-152.

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

Lynn, D. J., R. Higgs, A. T. Lloyd, C. O'Farrelly, V. Hervé-Grépinet, Y. Nys, F. S. Brinkman, P. L. Yu, A. Soulier, and P. Kaiser. 2007. Avian beta-defensin nomenclature: a community proposed update. Immunol. Lett. 110:86-89.

Ramasamy, K. T., P. Verma, and M. R. Reddy. 2012. Differential gene expression of antimicrobial peptides β-defensins in the gastrointestinal tract of *Salmonella* serovar Pullorum infected broiler chickens. Vet. Res. Commun. 36:57-62.

Röhe, I. 2014. Effects of feed structure on animal performance, gastrointestinal morphology, gut-associated lymphoid tissue and jejunal glucose transport in laying hens. Ph.D. Dissertation. Freie Universität Berlin.

Smith, K. G., and J. L. Hunt. 2004. On the use of spleen mass as a measure of avian immune system strength. Oecologia 138:28-31.

Su, S., K. B. Miska, R. H. Fetterer, M. C. Jenkins, and E. A. Wong. 2015. Expression of digestive enzymes and nutrient transporters in *Eimeria*-challenged broilers. Exp. Parasitol. 150:13-21.

Sunkara, L. T., W. Jiang, and G. Zhang. 2012. Modulation of antimicrobial host defense peptide gene expression by free fatty acids. PLoS One 7:e49558.

Trebichavský, I., and I. Šplíchal. 2006. Probiotics manipulate host cytokine response and induce antimicrobial peptides. Folia Microbiol. 51:507-510.

van Dijk, A., M. Herrebout, M. H. G. Tersteeg-Zijderveld, J. L. M. Tjeerdsma-van Bokhoven, N. Bleumink-Pluym, A. J. M. Jansman, E. J. A. Veldhuizen, and H. P. Haagsman. 2012. Campylobacter jejuni is highly susceptible to killing by chicken host defense peptide cathelicidin-2 and suppresses intestinal cathelicidin-2 expression in young broilers. Vet. Microbiol. 160:347-354.

Van Dijk, A., E. Molhoek, F. Bikker, P.-L. Yu, E. Veldhuizen, and H. Haagsman. 2011. Avian cathelicidins: Paradigms for the development of anti-infectives. Vet. Microbiol. 153:27-36.

Van Dijk, A., M. H. G. Tersteeg-Zijderveld, J. L. M. Tjeerdsma-van Bokhoven, A. J. M. Jansman, E. J. A. Veldhuizen, and H. P. Haagsman. 2009. Chicken heterophils are recruited to the site of *Salmonella* infection and release antibacterial mature Cathelicidin-2 upon stimulation with LPS. Mol. Immunol. 46:1517-1526.

van Dijk, A., E. J. A. Veldhuizen, and H. P. Haagsman. 2008. Avian defensins. Vet. Immunol. Immunopathol. 124:1-18.

Wang, G. 2013. Database-guided discovery of potent peptides to combat HIV-1 or superbugs. Pharmaceuticals 6:728-758.

Weintraut, M. L., S. Kim, R. A. Dalloul, and E. A. Wong. 2016. Expression of small intestinal nutrient transporters in embryonic and posthatch turkeys. Poult. Sci. 95:90-98.

Yang, L., T. M. Weiss, R. I. Lehrer, and H. W. Huang. 2000. Crystallization of antimicrobial pores in membranes: magainin and protegrin. Biophys. J. 79:2002-2009.

Zanetti, M. 2005. The role of cathelicidins in the innate host defenses of mammals. Curr. Issues Mol. Biol. 7:179-196.

Zhang, G., and L. T. Sunkara. 2014. Avian antimicrobial host defense peptides: from biology to therapeutic applications. Pharmaceuticals 7:220-247.

Table 2. qRT-PCR primers for turkey host defense peptides

Name	Forward primer (5'→3')	Reverse primer (5'→3')	Accession no.
GAPDH	GCTGAGAATGGGAAACTTGTGAT	GGGTTACGCTCCTGGAAGATAG	NM_001303179.1
AvBD1	TGTTTACGTCGGAATGGCTTCT	CTTGAACATGTCCCACTGATGAC	AF033337.1
AvBD2	AGAGGGACATGTTGTTCTGTAAAAGA	AACCCAAAGCAGCTTCCAACT	XM_003204631.2
AvBD8	AGCAGGCAGGAGGGATCTG	CGCCCAAAGGCTCTGGTA	XM_003204627.1
AvBD9	TGGCTCCTGCTCTTTTATTGC	AGCACTTCAGCTTCCCATCAC	XR_792753.1
AvBD10	TGCTCCAGGCTCAGCAGAT	CGGCAGAAATTCCCCTGATT	XR_792752.1
AvBD13	CAGCTGTGCAGGAACAACCA	CAGCTCTCCATGTGGAAGCA	G5E7L1
Cath2	GACACCCCCGAGATCAATCTAC	TCAGGAAGCGGCCAAATC	XM_003206909.2
Cath3	TGGACTCCATGGCTGATCCT	TCTTGATGGCTTTGTAGAGGTTGA	XM_010712309.1
LEAP-2	GATGACGCCCTTCTGGAGAG	CGGAGGCCGTTCTAAGGAAG	HGNC:29571

Primers designed by Primer Express v3.0 software (Applied Biosystems, Foster City, CA)

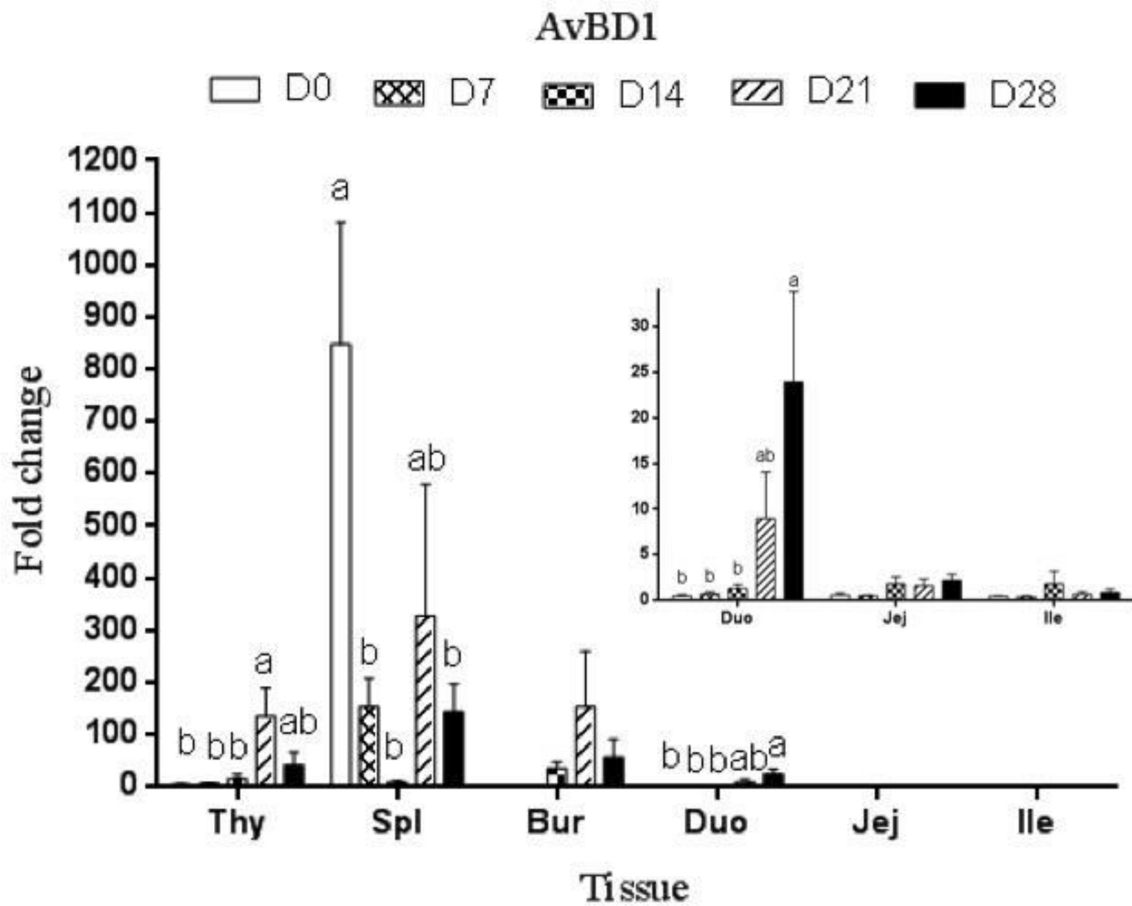

Figure 1. AvBD1 expression at different developmental ages. Fold change was determined using real time PCR with GAPDH as the reference gene. Tissues analyzed were thymus (thy), spleen (spl), bursa (bur), duodenum (duo), jejunum (jej) and ileum (ile). Bars represent means + SEM (n=6) for fold change for male turkeys at day of hatch (D0), D7, D14, D21 and D28. One-way ANOVA was run within a tissue. Bars with a different letter (a-b) per tissue are significantly different (P<0.05) when analyzed with Tukey's test.

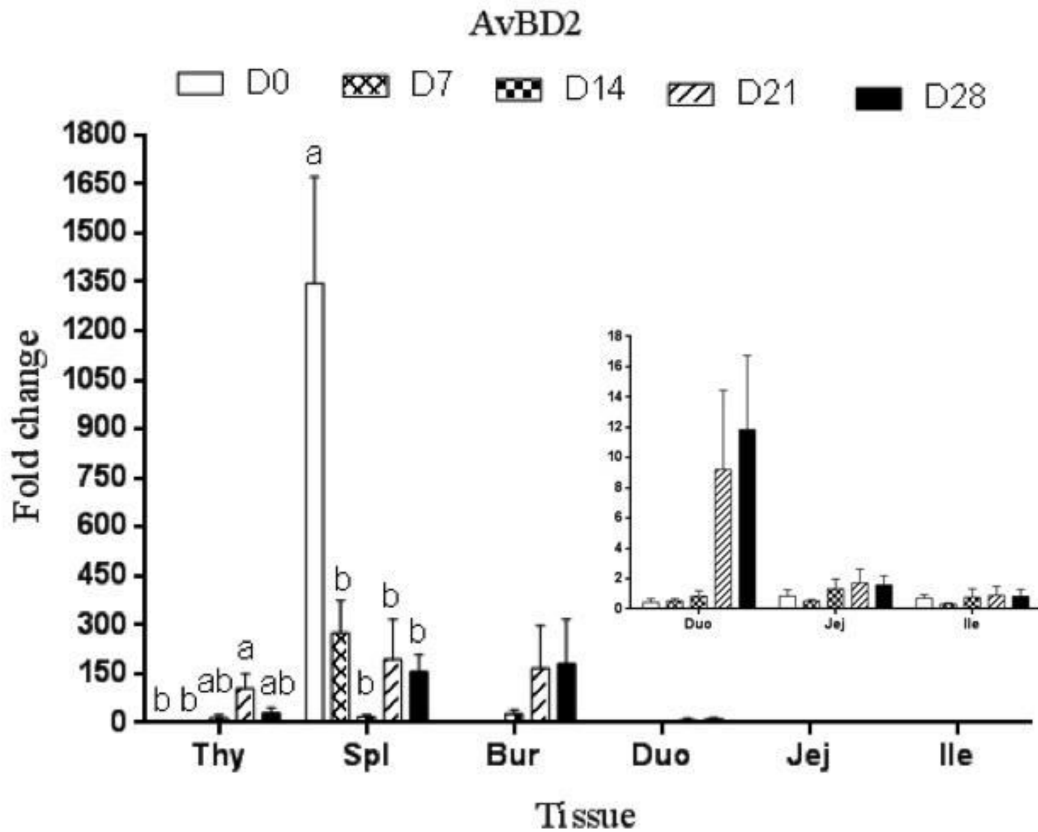

Figure 2. AvBD2 expression at different developmental ages. Fold change was determined using real time PCR with GAPDH as the reference gene. Tissues analyzed were thymus (thy), spleen (spl), bursa (bur), duodenum (duo), jejunum (jej) and ileum (ile). Bars represent means + SEM (n=6) for fold change for male turkeys at day of hatch (D0), D7, D14, D21 and D28. One-way ANOVA was run within a tissue. Bars with a different letter (a-b) per tissue are significantly different ($P<0.05$) when analyzed with Tukey's test.

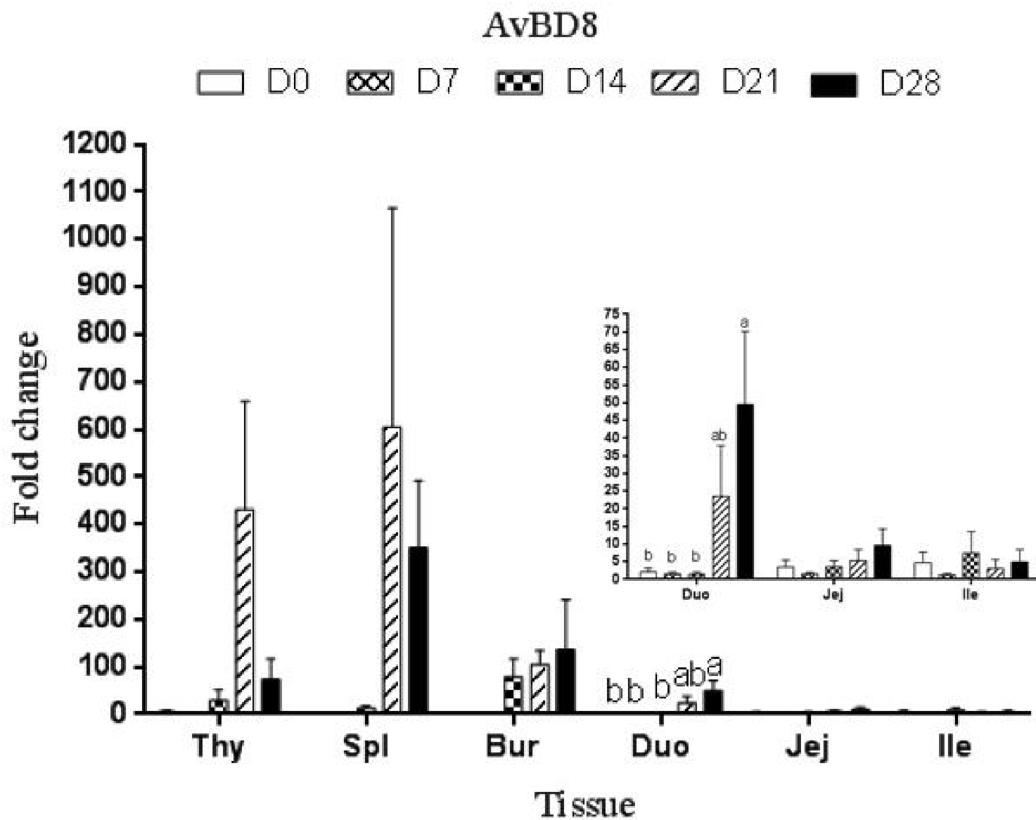

Figure 3. AvBD8 expression at different developmental ages. Fold change was determined using real time PCR with GAPDH as the reference gene. Tissues analyzed were thymus (thy), spleen (spl), bursa (bur), duodenum (duo), jejunum (jej) and ileum (ile). Bars represent means + SEM (n=6) for fold change for male turkeys at day of hatch (D0), D7, D14, D21 and D28. One-way ANOVA was run within a tissue. Bars with a different letter (a-b) per tissue are significantly different ($P<0.05$) when analyzed with Tukey's test.

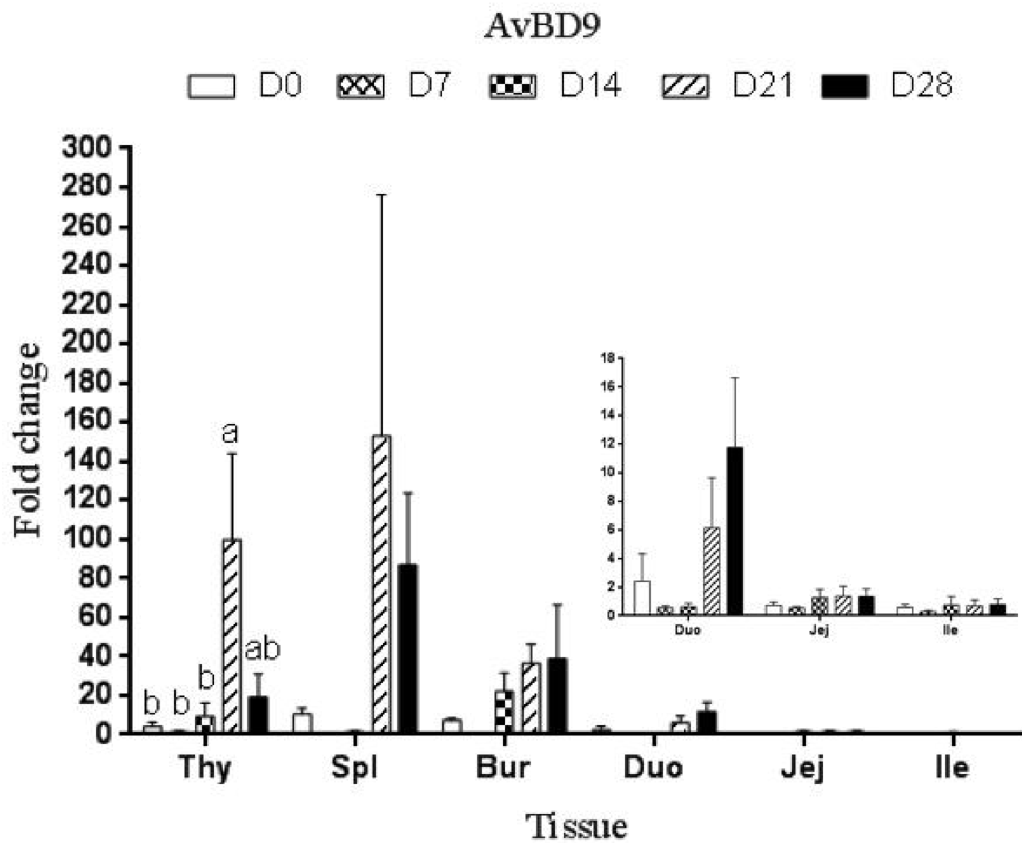

Figure 4. AvBD9 expression at different developmental ages. Fold change was determined using real time PCR with GAPDH as the reference gene. Tissues analyzed were thymus (thy), spleen (spl), bursa (bur), duodenum (duo), jejunum (jej) and ileum (ile). Bars represent means + SEM (n=6) for fold change for male turkeys at day of hatch (D0), D7, D14, D21 and D28. One-way ANOVA was run within a tissue. Bars with a different letter (a-b) per tissue are significantly different (P<0.05) when analyzed with Tukey’s test.

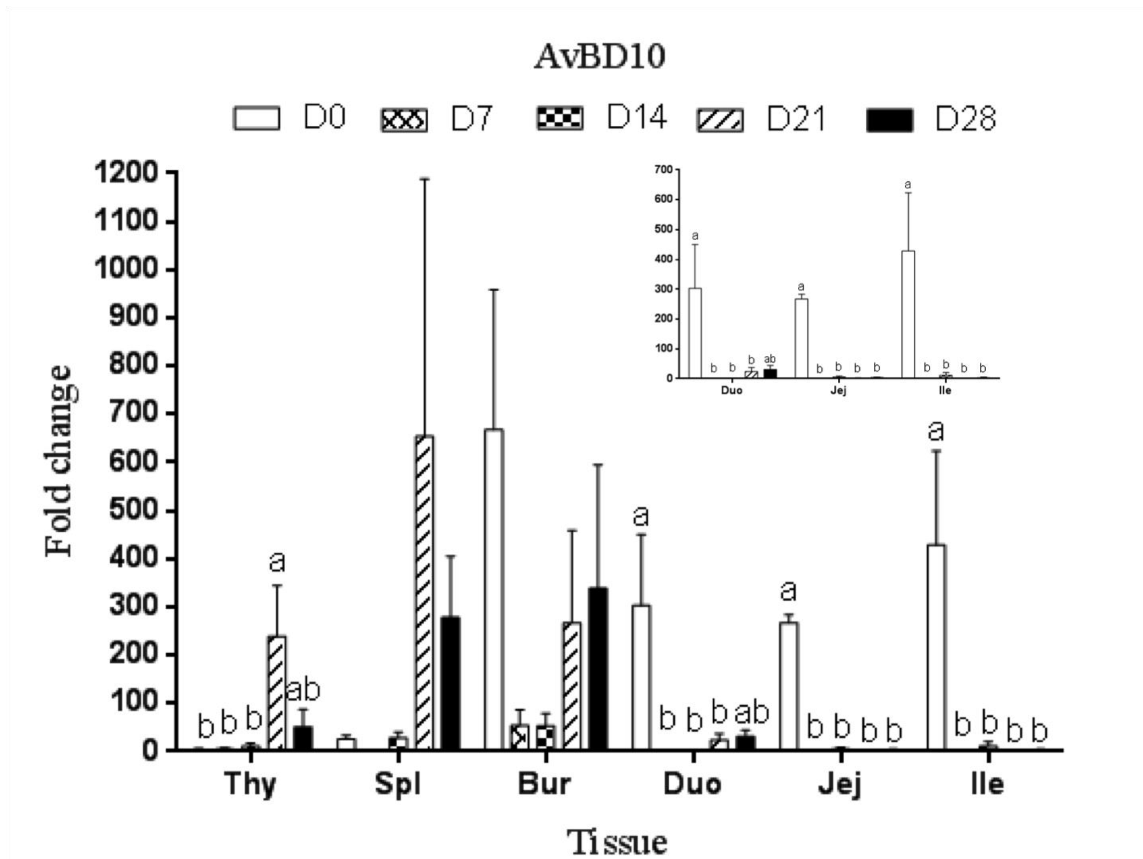

Figure 5. AvBD10 expression at different developmental ages. Fold change was determined using real time PCR with GAPDH as the reference gene. Tissues analyzed were thymus (thy), spleen (spl), bursa (bur), duodenum (duo), jejunum (jej) and ileum (ile). Bars represent means + SEM (n=6) for fold change for male turkeys at day of hatch (D0), D7, D14, D21 and D28. One-way ANOVA was run within a tissue. Bars with a different letter (a-b) per tissue are significantly different ($P<0.05$) when analyzed with Tukey's test.

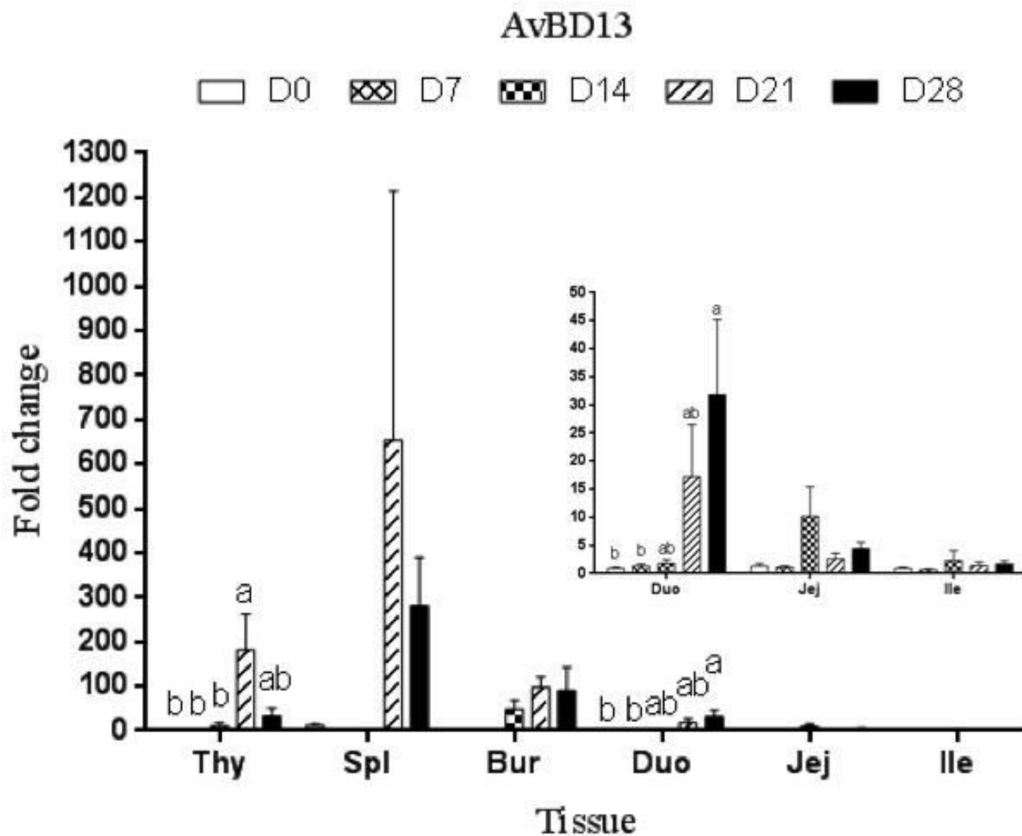

Figure 6. AvBD13 expression at different developmental ages. Fold change was determined using real time PCR with GAPDH as the reference gene. Tissues analyzed were thymus (thy), spleen (spl), bursa (bur), duodenum (duo), jejunum (jej) and ileum (ile). Bars represent means + SEM (n=6) for fold change for male turkeys at day of hatch (D0), D7, D14, D21 and D28. One-way ANOVA was run within a tissue. Bars with a different letter (a-b) per tissue are significantly different ($P<0.05$) when analyzed with Tukey's test.

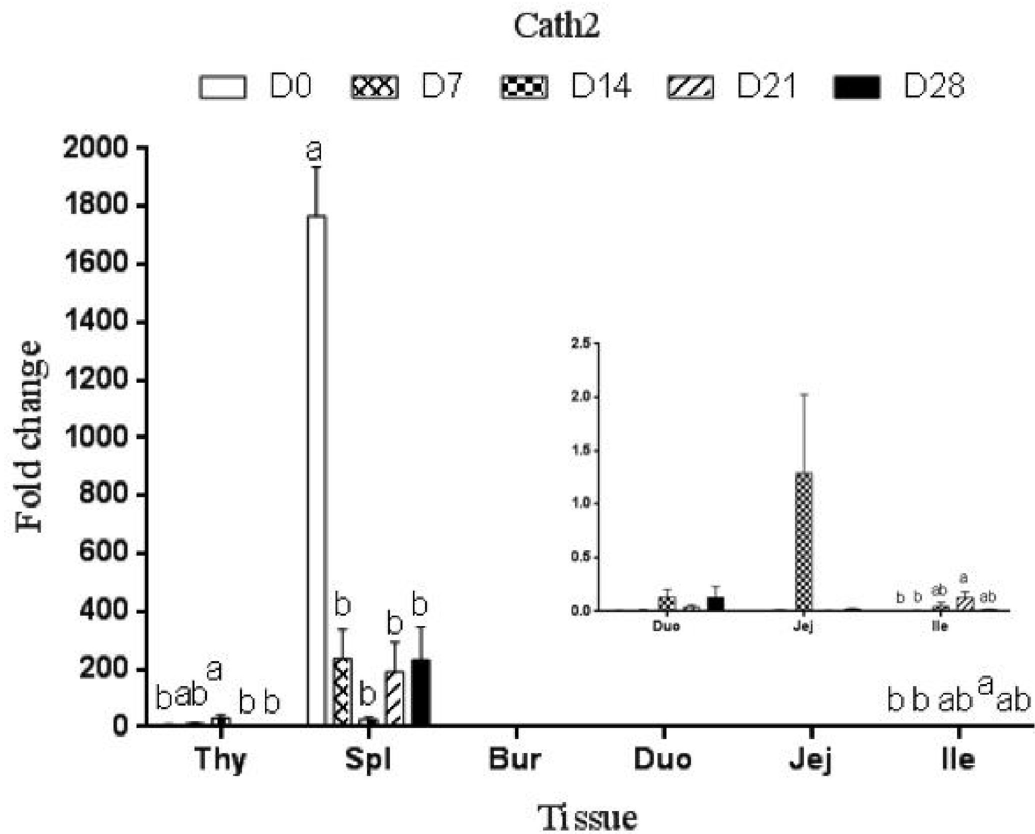

Figure 7. Cath2 expression at different developmental ages. Fold change was determined using real time PCR with GAPDH as the reference gene. Tissues analyzed were thymus (thy), spleen (spl), bursa (bur), duodenum (duo), jejunum (jej) and ileum (ile). Bars represent means + SEM (n=6) for fold change for male turkeys at day of hatch (D0), D7, D14, D21 and D28. One-way ANOVA was run within a tissue. Bars with a different letter (a-b) per tissue are significantly different (P<0.05) when analyzed with Tukey's test.

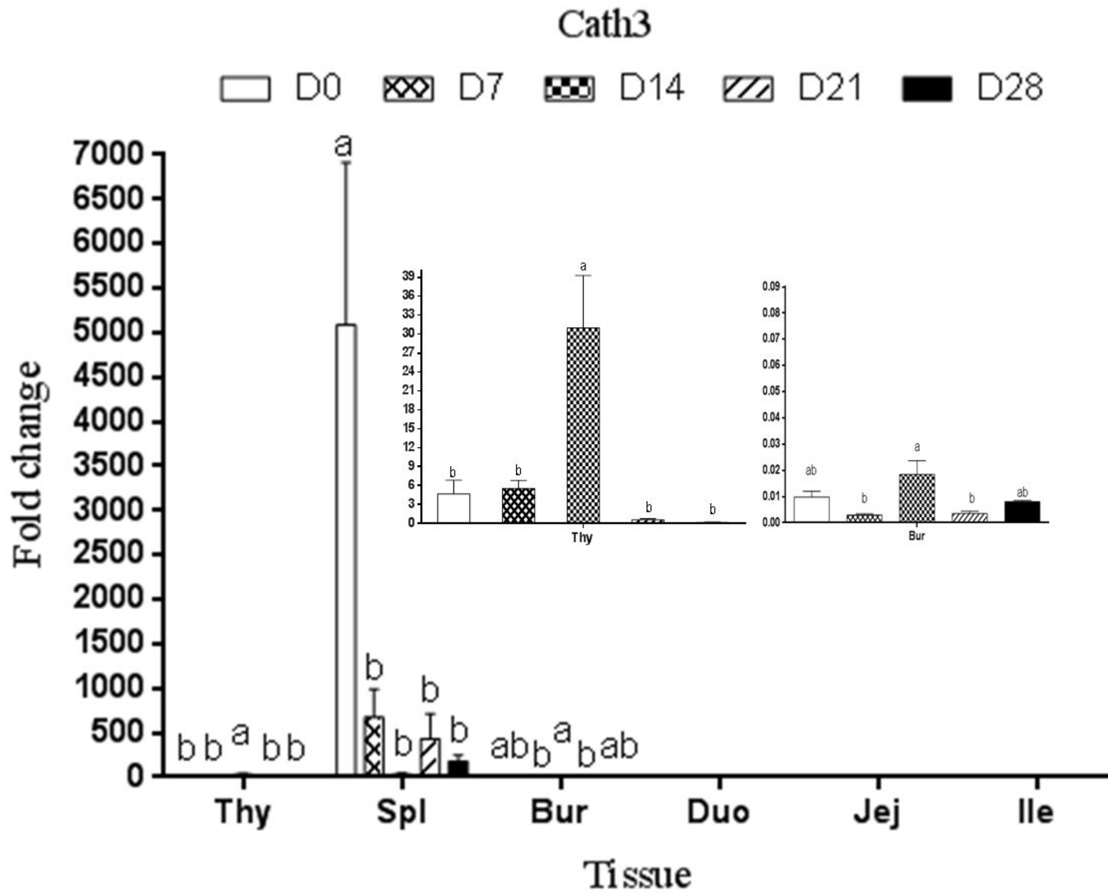

Figure 8. Cath3 expression at different developmental ages. Fold change was determined using real time PCR with GAPDH as the reference gene. Tissues analyzed were thymus (thy), spleen (spl), bursa (bur), duodenum (duo), jejunum (jej) and ileum (ile). Bars represent means + SEM (n=6) for fold change for male turkeys at day of hatch (D0), D7, D14, D21 and D28. One-way ANOVA was run within a tissue. Bars with a different letter (a-b) per tissue are significantly different ($P<0.05$) when analyzed with Tukey's test.

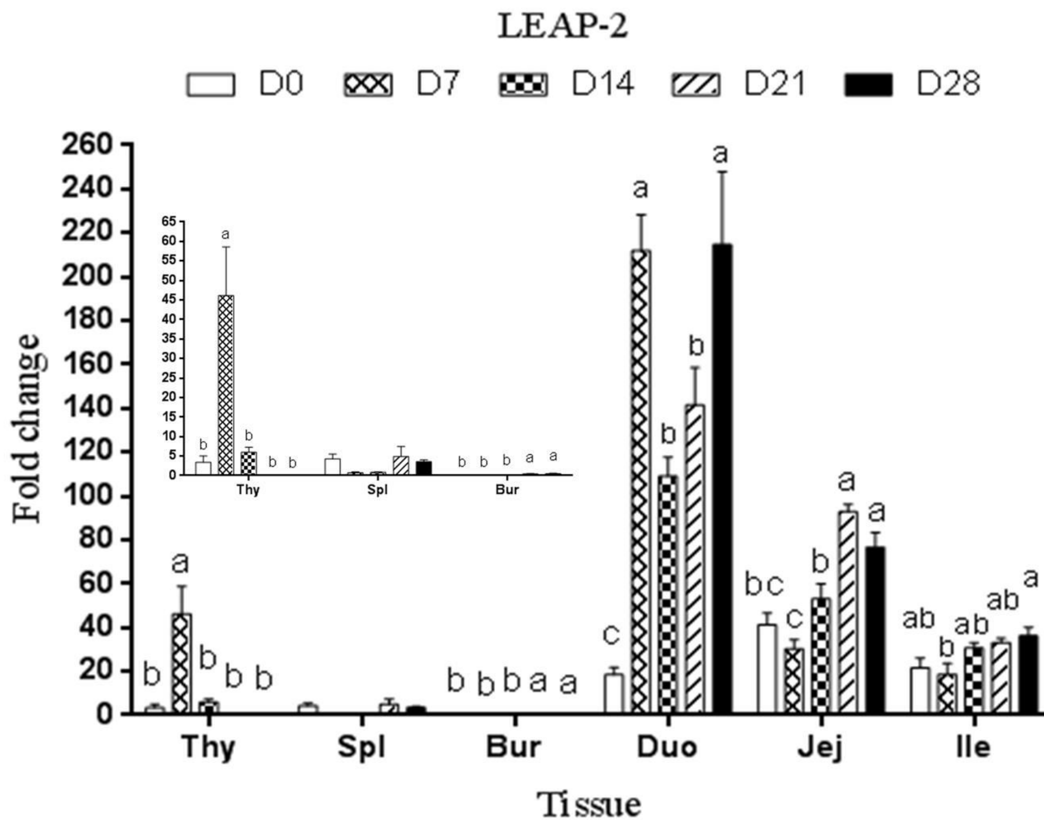

Figure 9. LEAP-2 expression at different developmental ages. Fold change was determined using real time PCR with GAPDH as the reference gene. Tissues analyzed were thymus (thy), spleen (spl), bursa (bur), duodenum (duo), jejunum (jej) and ileum (ile). Bars represent means + SEM (n=6) for fold change for male turkeys at day of hatch (D0), D7, D14, D21 and D28. One-way ANOVA was run within a tissue. Bars with a different letter (a-c) per tissue are significantly different (P<0.05) when analyzed with Tukey's test.

CHAPTER IV

EPILOGUE

Costs for the poultry industry are increasing and that makes studying immunology more important to avoid additional expenses due to treatment of sick birds, which often cannot defend against invading pathogens at young ages when some components of the immune system have not fully developed. In the U.S., the demand for turkey continues to rise and more research focusing on turkey health and diseases is needed. Turkey poults are of higher concern since they are more susceptible to disease.

This study sheds new light on the mRNA expression patterns of host defense peptides (HDPs) in turkey poults in six different tissues (thymus, spleen, bursa, duodenum, jejunum, and ileum) at five early ages (D0, D7, D14, D21, and D28). The nine investigated HDP genes were AvBD1, AvBD2, AvBD8, AvBD9, AvBD10, AvBD13, Cath2, Cath3 and LEAP-2. The developmental expression pattern is different between HDP classes (AvBDs, Caths and LEAP-2), but is similar within the class. In addition, the expression pattern of HDPs in turkey poults is generally similar to their counterparts in chickens. To our knowledge, HDPs have not been well studied in avian species other than chickens, so this project represents the first report of their expression in turkey poults.

This study of tissue and developmental expression of HDPs would be used as a guide to knowing the status of innate immunity of turkey poults, an important protein source especially in the U.S. The current study is a first step for understanding HDP expression, which may help in future development of new therapeutic strategies by promoting their expression to strengthen the innate immune response. This could be particularly useful for turkey poults during the first

stages of life when some components of their immune system are not fully developed and the birds are more susceptible to pathogens. The outcome of this study may provide baseline information for further research exploring new strategies to enhance the immune system of poults or finding alternatives for antibiotics. The main issue of antibiotics is the development of microbial resistance upon prophylactic applications, rendering their use ineffective for treatment purposes. Recent research investigated the potential of HDPs as antibiotic alternatives through inducing HDP expression by feed additives like yeast β-glucans, which were shown to induce HDP expression and improve the performance and health of chickens challenged with *Clostridium perfringens* (Tian et al., 2016). Also, knowing the expression pattern of HDPs may help in the genetic selection of birds with more robust innate immunity. Further research is needed to understand the actual role for each HDP in defending against pathogens, the specificity to common turkey pathogens, the actual mechanisms of these peptides in turkeys, and the structure-activity relationship of HDPs in turkeys.